



# MINERAL METABOLISM

BY

ALFRED T. SHOHL, M.D.

RESEARCH ASSOCIATE IN PEDIATRICS  
HARVARD UNIVERSITY



American Chemical Society

Monograph Series *52*

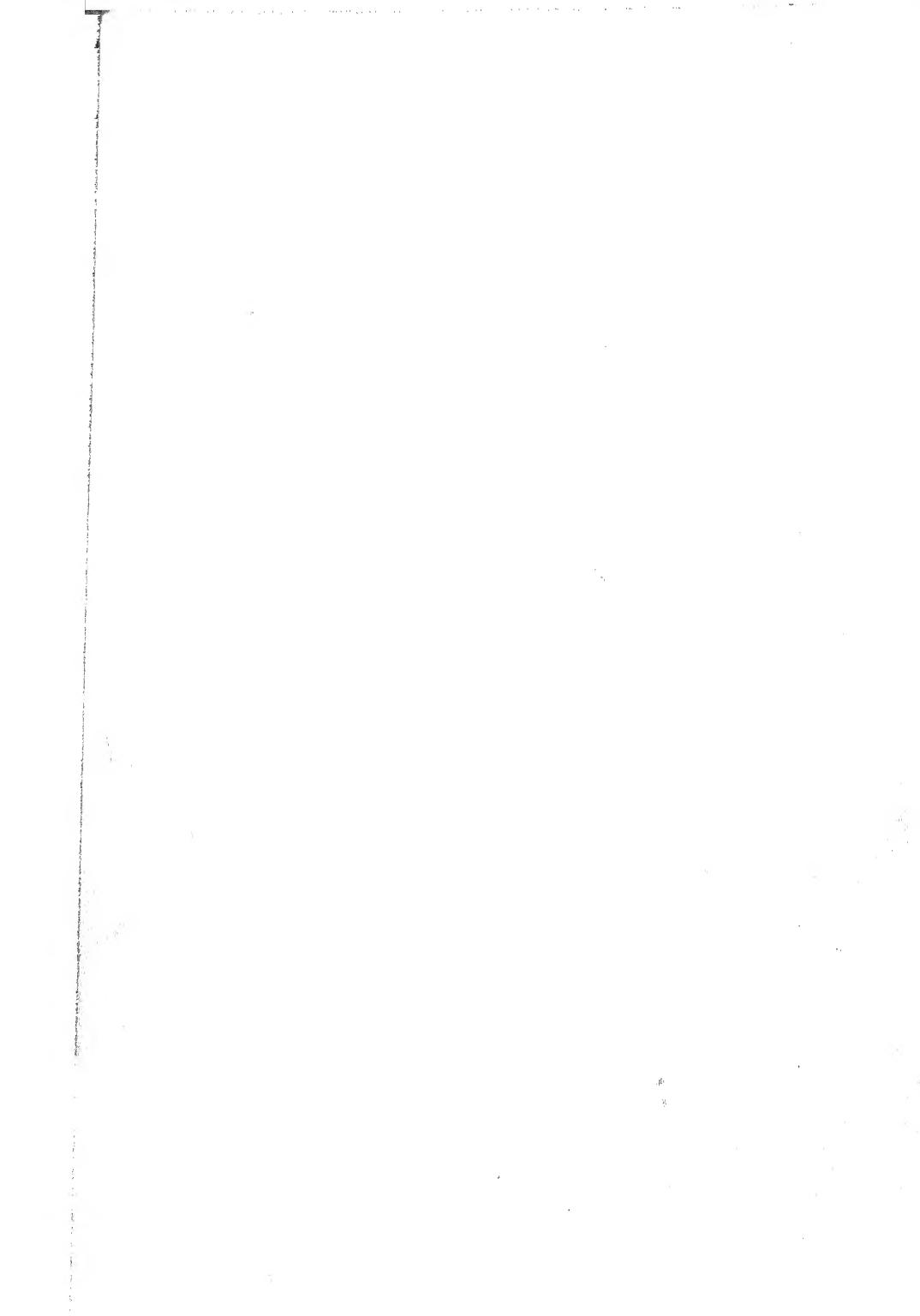
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## GENERAL INTRODUCTION

### American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the society, Washington, D. C.; the late John E. Teeple, then treasurer of the society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A. C. S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed editors (the present list of whom appears at the close of this introduction) to have charge of securing authors, and of considering critically the manuscripts submitted. The editors endeavor to select topics of current interest, and authors recognized as authorities in their respective fields.

The development of knowledge in all branches of science, especially in chemistry, has been so rapid during the last fifty years, and the fields covered by this development so varied that it is difficult for any individual to keep in touch with progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and by such compendia as Beilstein's *Handbuch der Organischen Chemie*, Richter's *Lexikon*, Ostwald's *Lehrbuch der Allgemeinen Chemie*, Abegg's and Gmelin-Kraut's *Handbuch der Anorganischen Chemie*, Moissan's *Traité de Chimie Minérale Générale*, Friend's and Mellor's *Textbooks of Inorganic Chemistry* and Heil-

bron's Dictionary of Organic Compounds, it often takes a great deal of time to coördinate the knowledge on a given topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value. It was with a clear recognition of the usefulness of such work that the American Chemical Society undertook to sponsor the publication of the two series of monographs.

Two distinct purposes are served by these monographs: the first, whose fulfillment probably renders to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a form intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far removed from their own. These monographs enable such men to form closer contact with work in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made, and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, extended references to the literature enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection is made of those papers which are most important.

## AMERICAN CHEMICAL SOCIETY

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DE SAUTY  
An Electrochemical Eclogue

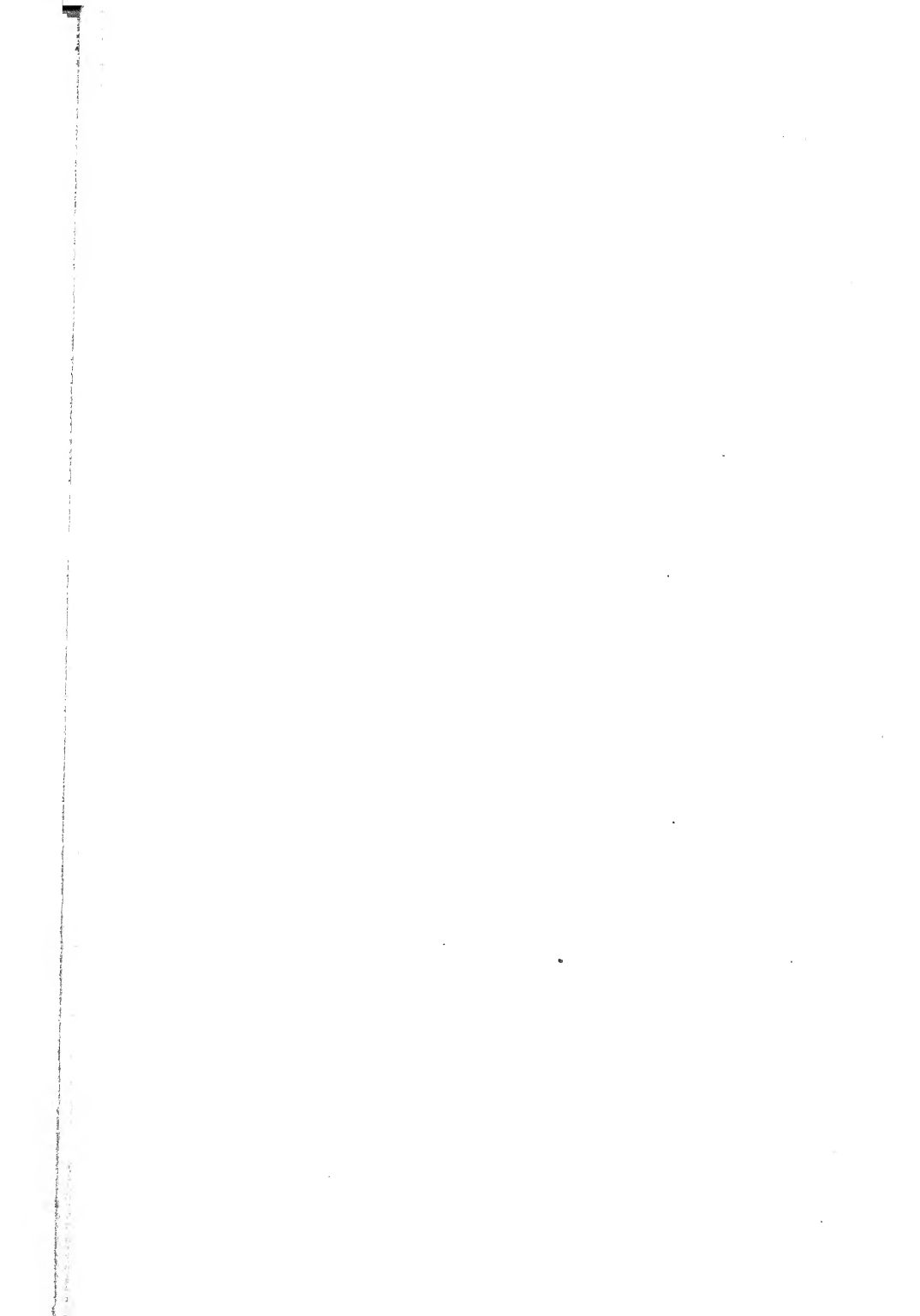
.....

Drops of deliquescence glistened on his forehead,  
Whitened round his feet the dust of efflorescence,  
Till one Monday morning, when the flow suspended,  
There was no De Sauty.

Nothing but a cloud of elements organic,  
C. O. H. N. Ferrum, Chlor. Flu. Sil. Potassa,  
Calc. Sod. Phosph. Mag. Sulphur, Mang. (?) Alumin. (?) Cuprum (?),  
Such as man is made of.

.....

From *The Professor at the Breakfast Table*, 1858-9.  
by Oliver Wendell Holmes.



## Preface

The main purpose of this book is to describe the role of the minerals in the structure and function of the human body. The separation of mineral metabolism from the context of physiology should not lead to distortion, but to clarity, for the physiology of the body as a whole can be understood only when there is knowledge of the many interrelated factors.

The field of mineral metabolism is at present in a phase of rapid expansion. A generation ago the center of interest in metabolism lay in calories and proteins. More recently the physiology of acid-base equilibrium and water metabolism have been placed upon a firm basis. Now the significance of traces or small quantities, not only of vitamins and other active organic substances, but also of minerals, is under intensive investigation.

The subject matter is presented as a connected account in brief form—a rational simplification and interpretation. The reader is assumed to have a knowledge of the background of biochemistry and physical chemistry. For the sake of continuity many aspects of mineral metabolism are omitted entirely and others only mentioned. It is hoped that this description will make available to the non-specialist the trends and meaning of a field in which much difficulty is caused by the great mass of conflicting data. In order to bring some order out of apparent chaos, a summary must necessarily lag behind the most recent investigations. A critical evaluation requires so much sagacity that this review makes no pretense to be exhaustive and, in those fields with which the author has only a bowing acquaintance, is inadequate. One is between the horns of the dilemma of writing more than he knows, or of being fragmentary. The latter is the better alternative.

Bibliography has been especially troublesome. No doubt certain advantage would accrue if more of the original citations had been given. But the literature is so voluminous that even if all the main articles could be included, the list would be out of date before it was published. The specialist does not need exhaustive bibliographical treatment, as he is familiar with the material in his field; and the layman in biology does not want to be bothered with much citation, but is more interested in obtaining a summary and perspective. Enough references have been given so that anyone interested can readily find access to the literature.

Thanks are due to various editors and publishers for permission to



reproduce certain tables and figures. I am under obligation to many for kindness, but especially to Dr. F. C. Bing, who has contributed the chapters *Iron* and *Iodine* and has also discussed the rest of the material and read critically all of the manuscript. Dr. W. M. Clark has critically reviewed the manuscript and has been most helpful in the deletion of errors and in the revision of terminology. I wish to thank also the friends who read and criticized various chapters: Drs. J. C. Aub, A. M. Butler, J. L. Gamble, G. P. Grabfield, A. B. Hastings, I. G. Macy, F. C. McLean, J. W. Mehl, H. F. R. Plass and W. C. Stadie. Mr. G. G. Hawley of the Reinhold Publishing Corporation has been most helpful in the preparation of the manuscript and the index. Finally, I am indebted to my wife, without whose constant encouragement and help in preparing the manuscript and bibliography this book could not have been written.

ALFRED T. SHOHL.

300 Longwood Ave.  
Boston, Mass.  
October, 1938.

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\*By F. C. Bing, Sec. of the Council on Foods of the American Medical Assoc., Asst. Prof. of Physiology, Northwestern University Medical School.

# Chapter 1

## Introduction

### Scope of Mineral Metabolism

Knowledge of the composition and requirements of the body has been constantly expanding during the last hundred years. It has become apparent that not only proteins, fats and carbohydrates, but also minerals and water are essential to life. More recently the significance of the vitamins in nutrition and the importance of the internal secretions in physiology have become known. These findings have served to emphasize the fundamental importance of the minerals by demonstrating the functions of some of them, or by offering improved opportunities to study experimentally the role of each mineral element in living processes.

At the time of Lavoisier only twenty-six elements were known. Sodium, potassium, calcium and magnesium were shortly thereafter separated by Davy. Chlorine had been isolated by Scheele; and phosphorus had been discovered in urine by Brand in 1669. Sulfur and iron had been known since prehistoric times, but knowledge of their occurrence in the body dates from the eighteenth century. Iodine was discovered by Gay-Lussac, but not demonstrated in the body. It is primarily to Liebig that we owe the appreciation of the importance of minerals as normal constituents of plant and animal tissues.

The elements which occur in biological material may be classed roughly in five groups.

1. C, H, O and N.

These four elements are grouped together because they occur in the organic components of tissues. They are found in carbohydrates and fats, and also in water, carbon dioxide and ammonia. The proteins contain all four of these elements and usually S and sometimes P.

2. Cl, P and S.

These elements form the important electronegative ions or ionogens—chloride, phosphates and sulfate—and are also found in important organic compounds in the body.

3. Na, K, Ca and Mg.

These are the electropositive elements of physiological importance.

## 4. Fe and I.

These two elements occur in small amounts, principally in well-defined compounds of fundamental importance, and therefore may well be classed together, separate from group 5.

## 5. Al, As, Br, Co, Cu, F, Mn, Ni, Se, Si and Zn.

These elements and others occur in traces, either in essential compounds or as contaminants.

The tissues of man and the known internal secretions and vitamins normally contain no elements not given in the foregoing list, and we are led to believe that this is true of the structure of the yet undiscovered substances.

**Definition of mineral metabolism.**—The terminology in this field of knowledge has never been entirely satisfactory, as the words *mineral* or *inorganic* do not define these elements or the compounds formed by them. The term *inorganic* might properly embrace all these elements, for carbon, hydrogen and oxygen are present in  $H_2O$ ,  $CO_2$ , carbonic and other acids, and nitrogen is present in inorganic form as ammonia, nitrite and nitrate. However, it has been customary to omit these from the list of mineral elements, although their inorganic compounds must be included in any discussion of mineral metabolism. Because the other elements are found largely in the ash of food and biological materials when burned in air, they have been grouped as ash constituents, minerals, mineral salts, inorganic elements or inorganic foodstuffs. They have also been designated as ions, fixed material, and in many other ways. However, the form of the material in the ash does not represent its condition before burning. For lack of a better term we have referred to all the elements in their simple inorganic forms as minerals.

The word *metabolism* also defies satisfactory definition. It has been used in relation to any and every phase of the changes of material in the life cycle of plants and animals, and therefore has a number of specific technical meanings. The term *mineral economy* used by the Germans has not been generally accepted. In the present account the term *mineral metabolism* is used in a broad sense to include the manifold activities of the minerals in life processes.

**Relation of minerals to water.**—At first glance the functions of the minerals are obscure. They yield no energy and may undergo no change in chemical combination in the body. It would appear, then, that minerals are needed only in growth, but such is not the case. The adult requires them as much as the infant. Probably every function of cellular activity is dependent upon the mineral elements.

Except in the skeleton where the salts are in the solid phase, all the compounds of the body, whether organic or inorganic, are present in watery suspension, in emulsion, or in true solution. Hence there is a

very close interrelation between water and the mineral elements. As shown by L. J. Henderson<sup>13</sup> in his classic work, "The Fitness of the Environment," water has unique and special characteristics which render it peculiarly efficient for biologic function.

The chemical processes that characterize living matter take place in ionized solution, the "milieu interieur" of Claude Bernard. Ions can be readily transported from one place to another and thus tend to be distributed. By virtue of the nature of solutes in water and their mutual ionization they stabilize and regulate many body reactions and equilibria, especially the ionic, osmotic and acid-base equilibria. The most striking example in physiology is the Ringer-Locke's solution, developed empirically before ionization was well known. Surviving tissues immersed in this solution maintain their physiological integrity for a long time. This solution is not only adjusted for the correct osmotic pressure (0.7 per cent NaCl for frogs, about 0.9 per cent for mammals) and acidity, but contains, in addition to  $\text{Na}^+$  and  $\text{Cl}^-$ , small proportions of  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{HCO}_3^-$ . The surviving heart immersed in simple solutions of  $\text{Na}^+$  and  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{Cl}^-$ , or  $\text{Ca}^{++}$  and  $\text{Cl}^-$  does not give a normal type of contraction, nor continue long to beat. This demonstrates the importance of the individual ion effects upon living tissue.

The functions of minerals are so important that they may well be said to control life itself. An account of the mechanisms of electrolyte action properly belongs to a treatise on general physiology, such as the monumental works of Bayliss,<sup>8</sup> Höber<sup>17, 18</sup> and the recent monographs of Abramson<sup>1</sup> and Loeb.<sup>22</sup> It is beyond the scope of this work to deal with these phenomena exhaustively, but it is impossible to discuss effectively the manifold problems of mineral metabolism except upon the physico-chemical basis.<sup>5, 6, 9, 14, 23</sup>

It has often been said that life had its origin in sea water. The ocean is in itself similar to a living organism. Material is constantly flowing into it and leaving it, but the ocean itself changes hardly at all. Its numerous chemicals are built up into various organic forms which, in time, are disintegrated and re-utilized. Like the body, the ocean maintains a constant osmotic, ionic and acid-base structure and a nearly constant temperature, and it uses for these purposes the same materials as those found in the body. The relation between  $[\text{H}_2\text{CO}_3]$  and  $[\text{HCO}_3^-]$  defines the acidity of sea water just as it does that of the body. The inorganic composition of sea water is, in general terms, similar to the composition of extracellular fluids in the body. Circumscribed bodies, such as the Mediterranean Sea, form almost as isolated compartments as the organs, and such currents as the Gulf Stream exert their influence in remote parts, as do the body secretions. In some respects even the ebb and flow of the tides with their inequalities in various places bring to

mind the increase and decrease of body fluids in different amounts in various systems.

The mineral composition of sea water is shown in the accompanying table:

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Cl <sup>-</sup>	CO <sub>3</sub> <sup>--</sup>	SO <sub>4</sub> <sup>--</sup>	Total positive	Total negative
Gm. kg.	11.0	0.4	0.4	1.3	19.0	0.09	2.7		
Meq. kg.	450	10	20	108	535	18	56	618	609

The concentration of the minerals in sea water is over three times that of the blood serum. There is reason to believe that this concentration has been increasing slowly during geologic times. Macallum has developed the interesting thesis, in an article called "Paleochemistry,"<sup>25</sup> that the mineral composition of the body fluids of animals reflects, in a general way, the composition of sea water at different geologic ages. Following Bunge and Quinton, he called attention to the similarity in the proportions of the several inorganic ions in serum and sea water, and suggested that the body fluids were derived from the sea. In mammalian serum, potassium is relatively high and magnesium and the total electrolyte concentrations are lower than those of sea water, facts which indicate the time in the evolution of the sea when the circulatory system was closed and became independent of its environment. The inorganic composition of the body fluids of other animals, such as *Limulus* with its high magnesium content, resembles more nearly the composition of the ocean at the present time. In other marine invertebrates, such as the lobster, the blood serum has the same relative composition as the sea water of pre-Cambrian days, but the concentration of electrolytes is about three per cent, and is a measure of the osmotic pressure of the ocean today.

This brief allusion to the interesting theory of Macallum emphasizes the fundamental relationships which exist between water and inorganic elements. In the body fluids, minerals occur in aqueous solution and the movement of electrolytes involves the movement of water, and *vice versa*. This subject is discussed more fully in Chapter 3.

**Relation of mineral metabolism to various fields of biology.**—The field of mineral metabolism properly embraces not only human physiology, but all living matter. A complete picture of mineral metabolism, accordingly, would include every phase of biology. In agriculture, for example, the addition of minerals to the soil is of fundamental practical importance. Such additions have been used empirically for centuries, but only since the time of Sir Humphry Davy have they been subject to scientific investigation. The last thirty years have witnessed great advances in the study of both the individual inorganic elements in fertilizers, and also of the importance of specific ionic effects and the acidity of the soil. Exact knowledge of these requirements has made possible

the recent development of water culture. Finally, the mineral metabolism of plants has become a special branch of botany, so great have been the developments in this field.<sup>19, 27, 35</sup>

In bacteriology, the preparation of artificial and purified media has necessitated the inclusion of salts in proper proportion and concentration for the growth of bacteria. The hydrogen-ion concentration of the media has proved to be as fundamental as the temperature, and studies show that not only is there a narrow zone of optimum acidity, but that growth occurs only near neutrality in slightly acid or alkaline environments. As a corollary, conditions more acid or alkaline are used to effect sterilization or disinfection. Pathogenic organisms grow in their living hosts in an environment of practically constant acidity and mineral concentration (the body fluids)—conditions close to optimal, either by selection or adaptation. In the intestinal tract alteration of acidity may be the factor determining the type of predominant organism or the invasion of the body by pathogenic bacteria. Such alterations may result from increase in fermentations or putrefactions, and depend upon the type of material present in the intestinal tract and the metabolic products of the bacteria. These products of bacterial metabolism may vary in the same organism. When sugars are present, the products are usually acid, and in absence of carbohydrate, alkali may predominate. These end-products must be disposed of by the body and may result either in acidosis or in alkalosis.

**Relation of minerals to animal nutrition.**—The feeding of domestic animals is of great economic importance. The theoretical principles involved are closely related to those of the nutrition of human beings but the problems are here intensified. The special requirements which vary according to the species are related to rapid growth, high fertility, and successful lactation. It is customary to feed farm animals mineral supplements to meet these needs. The large literature on animal nutrition is not cited, except in special cases, because the foodstuffs are so different from those for human consumption.

The mineral content of experimental diets became important as soon as an attempt was made to feed so-called synthetic diets to laboratory animals. Fifty years ago Forster<sup>11</sup> fed purified salt-poor diets and found that dogs and pigeons died in a few weeks. He thought that this confirmed the previously accepted opinion that minerals are necessary for life. As a matter of fact, because the diets used were relatively purified, the animals succumbed to a deficiency of vitamins. Lunin,<sup>24</sup> in extending Forster's work, first with the addition of sodium bicarbonate and later of other salts, realized that the salt was not the only factor, but stated that no other explanation was available at the existing state of knowledge.



Bunge<sup>4</sup> advanced the thesis that the mineral composition of the body closely resembled the mineral content of the milk of that species. This carries with it the corollary that the minerals in milk are present in optimal proportion and concentration. Lunin used the ash of milk as his guide when he attempted to supply salts to make his purified salt-free diets "complete." Osborne and Mendel,<sup>30</sup> in their classic studies upon the nutritive value of amino-acids, found that rats could be made to live only when "protein-free milk" was added to a mixture of purified protein and certain fats. Their salt mixture IV was based upon the analysis of protein-free milk and, with added lactose, was called "artificial protein-free milk." This resulted in cessation of growth sooner than with natural protein-free milk. After it was realized that protein-free milk carried vitamins as well as minerals, the vitamins were considered separately, but the mineral content of milk continued to serve as the basis of their salt mixture. McCollum<sup>28</sup> made an exhaustive study of the mineral content of various seeds. Salt mixtures comparable to those found in seeds and grains resulted in nutritive failure. He came to the conclusion that they were deficient in calcium, sodium and chlorine. He reported much better success with salt mixtures made in imitation of the inorganic content of milk or egg. Cowgill<sup>7</sup> used the analysis of urine as a guide when he devised a salt mixture suitable for adult dogs.

Practically all subsequent investigators have based their procedure on that suggested by Bunge, in the preparation of purified diets; they use salt supplements in the amount and proportion of minerals in milk. Experience demonstrates that successful nutrition can be obtained when adequate salt mixtures are fed at a level of about four per cent of the dry diet.<sup>31</sup>

Animal experiments offer many advantages. Heredity can be uniform, previous nutrition determined and pathological material eliminated. The variables can be reduced to a minimum and experiments can be controlled by use of litter mates and the paired feeding method. Moreover, the shorter life span of laboratory animals makes possible experiments covering the whole life or several generations. Further, they may be subjected to extreme conditions which make results clear cut. The conclusions from experiments with one species, however, cannot be applied directly to another, for although analogous, each species differs from others in many respects. Quantitative data applicable to man must be sought from experiments on human beings.

### Previous Reviews

Sporadic experiments on the mineral metabolism of man were made early in the history of modern physiology. Among the first of impor-

tance were the investigations of Schmidt in 1850 on the mineral exchanges in Asiatic cholera, and those of the Dorpat school on the effects of acids and alkalis. It was not until 1881 that a comprehensive review appeared, by Voit.<sup>36</sup> Bunge devoted the major part of his scientific life to investigations on the significance of minerals and laid the groundwork in many fields. His viewpoints were summarized in his textbook.<sup>4</sup> Noorden<sup>29</sup> realized the application to medical problems of this branch of physiology. The first separate monograph was that of Albu and Neuberg<sup>2</sup> in 1906, and this remains a classic. Wendt<sup>37</sup> published a review in 1911 and revised it in 1925.

The first general review of mineral metabolism in this country was written by Mattill and Mattill in 1922.<sup>26, 34</sup> The most complete information concerning mineral metabolism in infancy and childhood is to be found in Czerny and Keller's monograph.<sup>8</sup> Sherman, through active experimental work and the widespread influence of his textbook, "The Chemistry of Food and Nutrition,"<sup>33</sup> has been a leader and mentor in this field. Peters and Van Slyke's book, published in 1931,<sup>32</sup> deals authoritatively and comprehensively with many aspects of the minerals and their application to physiological pathology. In the same year Klinke's excellent monograph appeared.<sup>20</sup> This contains, in addition to a section on physiology, material on theory and application to pathological conditions. Both of the last mentioned monographs have dealt sparingly with the mineral composition of the body and its implications, and with the mineral intake, output and balances. This latter aspect of the problem is relatively new, as attested by the fact that the first complete mineral balance study of infants was made by Blauberg in 1900, and of adults by Wendt in 1905.

### Plan of This Review

Without going into such philosophical questions as the meaning of organization or the nature of vital processes, a simple description of the role of the minerals in the human machine as we find it functioning is attempted. The material in this review is selected from data on human beings, and the important results of other investigations are used only to supplement such data. The normal physiology of the body is the main interest, and pathological material is used principally to illustrate the functioning of mechanisms under stress. Disease is not discussed from the viewpoint of therapy, nor from that of a treatise upon medicine. Pathologic physiology offers insight into the mechanisms of mineral metabolism under the conditions of the experiments which nature performs daily.

The older expositions of mineral metabolism were organized under the headings of the individual elements. All the data concerning calcium,

for example, were grouped in one place, thus giving a general survey of its functions. But this method has drawbacks, for more than one element is operative in almost all the activities of the organs and tissues. Hence, when described as the action of single elements, the material assumes a degree of simplicity and specificity which it does not possess, and to that extent gives a false conception. Increasing knowledge has revealed that the relations of the elements to one another, to water, to acid-base equilibrium, and to body functions are so complicated that such a scheme no longer seems adequate. Thus one is faced with the alternative of discussing many physiological functions under each element or several elements under each function. The latter method has been adopted in the main. Thus the material on each element is widely scattered throughout the book, and only brief summaries and individual problems included in the chapters on the separate elements.

The first subject of discussion is the mineral composition of the body as a whole and of its several parts. This discloses both the qualitative and quantitative distribution of minerals. The body is composed of diverse systems, which are dependent for their activities on their composition. These complex biological systems have many interrelated regulatory devices, and the role of the minerals in these mechanisms is depicted. Material must constantly be brought to the body, utilized and disposed of, to keep it intact. Because the body functions without intermission, the losses of wear and tear must be repaired continuously. Material must be supplied not only for energy expenditure and replacement, but also for growth. Therefore in the concluding chapters the relation of the minerals in food to their utilization, storage, excretion and wastage is reviewed.

Much remains to be learned of the fundamental mineral structure of the body. Except for a few of the components of the blood, it has been impossible to give more than average values. It is to be hoped that future writers can present a better insight into the physiological constants. Modern micro-methods are subject to wide fluctuations in the degree of accuracy, depending upon both the method and the expertness of the investigator. Widely divergent data are encountered and the reviewer cannot always discern the source of error. Methods must be designed for the particular problem involved; there is always the question of the accuracy obtainable and that necessary to show significant differences. Failure to understand such fundamentals leads to the reporting of many data with non-significant figures. Normal variation should be known and stated statistically in terms of standard deviation. The difficulty of obtaining suitable data is due both to biological variation and to variations in experimental conditions, known and unknown. The author has made decisions as to the validity of reported values with

these problems in mind. The reader must consult the original data in regard to his specific problems.

### Terms and Definitions

Because of the considerable confusion and laxity in the use of terms it is necessary to present and define those employed in this book. The current physiological literature contains many terms which are not chemically correct, and hence are misleading. We have not eliminated these completely, but have made certain compromises with present usage. At the suggestion of Dr. W. M. Clark we have attempted to clarify the terminology by the use of expressions more acceptable to chemists.

Electronegative and electropositive elements or radicals are referred to as such in this book, or are called *anions* or *anionogens*, and *cations* or *cationogens*, respectively. The suffix *-ogen* expresses the fact that the elements which are non-ionized become ions in the course of body processes. Unfortunately the terms *acid* and *base* have been used for the above frequently in the physiological literature, in dealing with acid-base equilibrium (e. g.,  $\text{Na}^+$  is called a *base* and  $\text{Cl}^-$  an *acid*). We have eliminated this usage.

Brönsted<sup>10a</sup> has employed *base* to mean a substance which has given up a proton; e. g.,  $\text{Cl}^-$  is a *base*. Although the Brönsted terminology has aroused considerable interest, its main advantage appears when it is applied to non-aqueous systems. His terminology has not been adopted in this text.

The term *acid-base equilibrium* is commonly used in the literature with at least four different meanings, only one of which is retained in this book. *Acid-base equilibrium* as here used refers to the relative amounts of the various species of anions and their acids present in blood and other body fluids, and the resulting pH number. For discussion see page 274.

The sum of all the electropositive elements or radicals must equal the sum of the electronegative elements or radicals to meet the demand of electroneutrality. We have referred to *electroneutrality* and have rejected the terms *acid-base equilibrium* or *acid-base balance* sometimes used for this relationship.

Analysis of the minerals in body fluids or tissues, expressed in equivalents, usually shows an excess of either mineral anionogens or cationogens. This stoichiometric value we have designated as *excess of mineral anionogens* or *cationogens*, occasionally abbreviating the expression to *excess anionogens*, etc. When discussing the mineral content of food we have used the term *acid-ash value* or *alkaline-ash value* in this sense. These values are sometimes erroneously called *acid-base equilibrium* or *acid-base balance*. For discussion see pages 290-292.

The excess or deficit of negative or positive minerals in the intake compared to that in the output is expressed, in this book, as *mineral*

*cationogen-excess balance*, sometimes abbreviated to *cationogen-excess balance*. We have not employed the commonly used terms *acid-base equilibrium*, *acid-base balance* or *base balance* as a definition of this relationship. For further discussion see page 300.

*Total base* is a term which is so well established and convenient that it has been retained in this book to denote the analytical value for the sum of the electropositive elements, Na, K, Ca and Mg. However, we have preferred the designation of this value as *total mineral cationogens*, or in abbreviated form as *cationogens*. The term *total mineral anionogens* is similarly used for the sum of the equivalent values of P, S and Cl. We have avoided the terms *fixed base* and *fixed acid* for these values.

There are certain salts and organic compounds which are altered or oxidized in the body and yield mineral anions but not mineral cations, and *vice versa*. To emphasize this fact we have sometimes used the term *acidogen* for such mineral anion-producing substances (e.g.,  $\text{NH}_4\text{Cl}$ ), and *alkaliogen* for such mineral cation-producing substances (e.g.,  $\text{NaHCO}_3$ ).

Strong electrolytes (e.g.,  $\text{NaHCO}_3$ ) in solution have been considered, in this book, only as cations and anions ( $\text{Na}^+$  and  $\text{HCO}_3^-$ ). The term *salt solution* has been discarded, and the word *salt* retained only in its analytical sense.

We have employed the term *proteinate* instead of *base bound to protein*, whether the substance is ionized or non-ionized.

The earlier workers expressed their data on the minerals in weights of oxides. These values were obtained largely by analysis of ashed material and represented the analysts' desire to have the sum of the parts equal the whole. The results of modern analyses are more often expressed as weights of the elements. A more informative method is the description of the minerals expressed as equivalents, in terms of their concentrations, a procedure which has been adopted in this book when possible. This review has not been written as an exposition of chemical thermodynamics, and therefore the activity coefficients have been used but sparingly. A great proportion of the literature covered does not lend itself to such treatment.

In dealing with solutions it is convenient to consider the number of mols of material dissolved in 1000 gm. of water instead of contained in 1 liter of solution. The former unit of measure has been adopted in the development of the modern physico-chemical theories of solutions. The term used by Lewis<sup>21</sup> for characterizing such solutions is *molal*, to distinguish it from the latter, which is known as *molar*. Small as this difference may seem, it becomes very large in the case of body fluids. For example, the  $[\text{K}^+]$  in blood cells is 110 meq./l. of cells, but 173 meq./1000 gm. of water contained in the cells. It is not the amount of the electrolytes per volume of cells and body fluids, but the amount per volume of

cell water and body fluid water which represents their effective concentrations.

Following are definitions of other terms used in this book:

*mol*, or *M* = the molecular weight in grams.

*molar solution* = 1 mol made up to 1 l. with water.

*molal solution* = 1 mol dissolved in 1000 gm. of water. We have used interchangeably the terms *per 1000 gm. of water* and *per l. of water*.

*equivalent*, or *eq.* = 1 mol  $\div$  the valence. Phosphate radicals are either univalent, divalent or trivalent, depending upon the acidity of the solution in which they occur or upon the compounds in solid phase. Hence the equivalent value varies with the conditions.

*normal solution*, or *N* = 1 gm. eq. made up to 1 l. with water.

*osmol*, or *osM* = the amount of electrolyte equivalent in osmotic pressure to that of a molar or molal solution of non-ionized solute.

*milli-*, or *m*, used as a prefix to mol or equivalent, written *mM* and *meq.*, denotes 0.001 of the amount, and as a prefix to molar, *osmolar*, etc., denotes 0.001 of the concentration. For example: 1 *meq.* = the amount of a substance contained in 1 cc. of *N* solution.

*microgram*, or  $\mu\text{g}$  = 0.000,001 gm., or .001 mg.

[ ] denote concentration.

The present discussion presupposes a background knowledge of the laws of osmotic pressure and electrolytic dissociation, which form the fundamental basis for thinking about chemical reactions.<sup>10, 12, 15, 19</sup> This is necessary for understanding the physiology of the minerals, which act through chemical mechanisms.

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## Chapter 2

# Mineral Composition of the Body

The chemical structure determines and limits the functions of the body, and therefore a study of the chemical anatomy throws considerable light upon physiology. This chapter comprises a summary of the composition of the body as a whole, of the organs, and of the body fluids, in regard to water and minerals. It includes description of both the qualitative and quantitative interrelationships of the electropositive and electronegative elements and radicals. Such description furnishes a background necessary to the understanding of the chemical mechanisms in health and disease, and under various conditions such as growth, adolescence, pregnancy and lactation, and states of abundance and scarcity.

### BODY WATER

The water metabolism of the body constitutes one of the fundamental problems of physiology. Upon it depend the conditions of the hydration of the colloids, the osmotic pressure, and the mechanism of heat regulation through evaporation. In addition, the water content influences the size of the body, and hence of the surface, of which heat dissipation is a function. These problems are discussed further under *Water Metabolism*. Water constitutes two-thirds of the weight of the body, and is the principal component from the anatomical as well as from the physiological viewpoint. The constancy of weight bears ample testimony to the rigor with which the body guards its water content, and the efficiency of the control mechanism. "Rubner early called attention to the fact that in starvation an animal can lose practically all of his glycogen and fat and half his body protein, approximately 40 per cent of body weight, whereas the loss of 10 per cent of the water content of the body results in serious disorders, and the loss of from 20-22 per cent results in death."<sup>92</sup>

The water content of emaciated and fat animals varies. Voit<sup>116</sup> calculated that about nine per cent of the body of a new-born infant and 18 per cent of that of an adult are fat. (Later investigations indicate that 12 per cent is a better value for the new-born). He showed that fat constitutes a "foreign body" which replaces water to only a slight extent, and that, when the fat is removed, fat and thin animals show essentially the same percentage of water. This thesis has been amplified



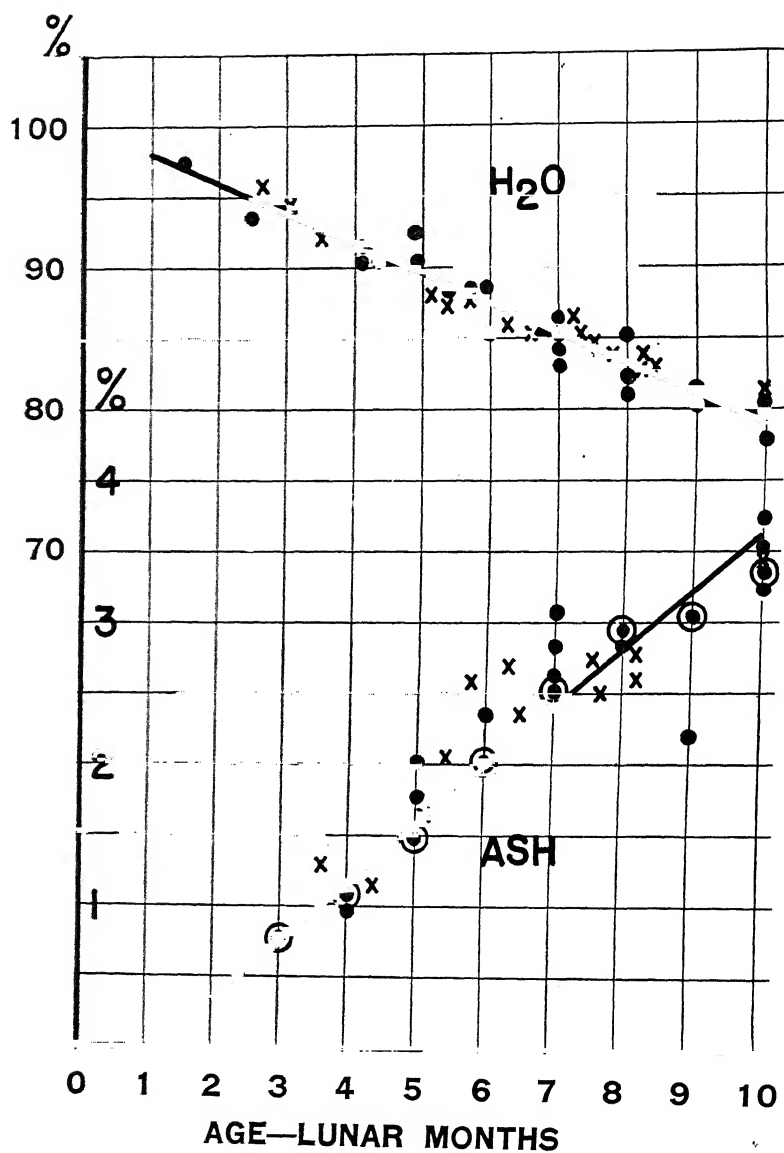


FIGURE 1. Ash and Water Content of the Fetus (Fat-free).\*

\* Calculated from the literature.

● = Summaries of the earlier work by Czerny and Keller.<sup>18</sup>

⊙ = Givens and Macy.<sup>22</sup>

x = Iob and Swanson.<sup>54</sup>

by Moulton<sup>2, 81</sup> for many different mammals. Needham<sup>84</sup> has given extensive data for the water content of embryos of many species.

Considerable reliable data exist on the water content of human fetuses and infants, but little on children or adults. In 1877 Fehling<sup>23</sup> analyzed the bodies of 21 fetuses. He dried the material to constant weight, extracted it with ether, and ashed the remainder. The material extracted by ether he called fat (the amount of fat in the fetus is negligible until the fifth month), and the difference between the residue and the ash he called protein. The six weeks old fetus contained 97.5 per cent water, and the new-born 74 per cent, or 81.5 per cent on a fat-free basis. It is apparent that the water percentage of the fetus decreases with age.

The earlier studies which have been reviewed in detail by Czerny and Keller<sup>18</sup> have been supplemented by more recent data.<sup>32, 54</sup> The results of more than a hundred analyses of the fetus, premature, new-born and infant have been compiled in a graph, Figure 1.

Table 1. The Weight and Water Content of Body Organs.\*

Organ	Weight (gm.)	Weight (% of total)	Water (gm.)	Water (%)	Water (% of total)
Skeleton .....	11080	16.0	(2442)	(22.0)	(6.1)
			5100†	46.0†	12.5†
Muscles .....	29112	41.6	22022	75.6	54.8
Intestines .....	1266	1.8	943	74.5	2.3
Liver .....	1576	2.3	1076	68.2	2.6
Spleen .....	131	0.2	99	75.7	0.2
Kidneys .....	259	0.3	214	82.7	0.5
Lungs .....	475	0.6	375	79.0	0.9
Heart .....	332	0.5	263	79.2	0.6
Brain and spinal cord....	1403	2.0	1050	74.8	2.6
Nerves .....	290	0.4	169	58.3	0.4
Skin .....	4850	7.0	3493	72.0	8.7
Fat .....	12570	18.0	3760	30.0	9.3
Blood‡ .....	3418	5.0	2836	83.0	7.0
	66762	95.7	41400	Av. 61	102.4

\* Bischoff's data of 1863 on a 69.7 kg. man, aged 33 yrs. Cited by Voit.<sup>116, p. 353.</sup>

† Data of A. W. Volkmann, cited by Klose.<sup>51</sup> This correction for skeletal water brings the value for the total water content into agreement with the known fact that the water content equals 70-76 per cent of the fat-free body weight, and varies with the fat content.

‡ Determination of blood volume in dead bodies is known to give extremely low values, because the blood cannot be removed from the organs.

• It is characteristic that prematures and new-borns show water loss during the first ten days of life. This is associated with a loss of minerals, especially sodium and chloride.

Bischoff<sup>5</sup> determined the water content of a new-born girl weighing 2.97 kg. He also determined the water content of the body of a 33-year

old man, 69.7 kg. in weight. These data are given in Table 1. In the infant he found 66 per cent water; in the adult 58 per cent. On a fat-free basis, calculating 12 and 18 per cent fat content, these values become 75 and 71 per cent respectively. He was not only the first to show the decrease in water content with age, but the first to determine the water content of the organs of the adult.

Cerebrospinal fluid contains more than 99 per cent of water, and the enamel of teeth as little as two per cent. Muscles contain about 70 to 75, the skeleton only 40 to 60, and adipose tissue still less—15 to 30 per cent. It is further obvious that the muscles, not only from their abundant content, but also from their great mass, form the water reservoir of the body, with over half of the total supply. Each of the organs constitutes a compartment in which are found solutions of minerals adapted to carry out different specific functions. The values for the organs of the adult only are known. It would be valuable to have similar data on the composition of the organs of the fetus and child, to determine whether these characteristics are altered with growth. Such material is available for the skin (see p. 33).

The question of the forms of water in the body will be considered later under *Water Metabolism*. For the purposes of the present discussion it will suffice to mention that the water has been considered only in its "free" form. The "bound" water or colloidal water is considered to represent a fraction which probably does not exceed two to four per cent, as estimated by Hill and others. Thus it is apparent that this constitutes a problem of little significance for the quantitative estimation of the dissolved minerals.

### Distribution of Body Water

Of the total water in the body some lies in the blood plasma and other circulating fluids—lymph, cerebrospinal fluid, etc.—and the rest occurs as that within the cells and that surrounding the cells. We owe to Gamble *et al.*<sup>29</sup> our insight into the significance of these various fractions of the total water. He made the fundamental hypothesis that from the known water and mineral composition of intracellular and intercellular fluid one could compute their volume changes in health and disease. Experimental data are rapidly accumulating which show the soundness of Gamble's judgment; these have proved useful in interpreting the mechanisms of edema, dehydration and urinary excretion. Peters has treated this subject recently in monographic form.<sup>86</sup> Discussion of the physiology of body water is deferred until more material concerning body structure has been presented. (See p. 45.) Gamble estimates that plasma water constitutes roughly 5 per cent, intercellular water 15 per cent and intracellular water 50 per cent of the body weight.

## ASH OF THE BODY

The ash obtained by incineration is the most comprehensive measure of the mineral content of both plant and animal material. Such analyses have been carried out for over a century. By this method incontestable evidence of the importance of minerals in animal and vegetable economy was produced by Prout, Boussingault, and Liebig. Albu and Neuberg<sup>1</sup> have pointed out that the ash does not accurately represent the original material, for chlorine, sulfur, sodium and potassium may be lost in ashing. Newer methods of ashing, such as that proposed by Stolte, or the use of uniform low heat in an electric muffle furnace, lead to better results. However, acid should be added to prevent the loss of positive elements, and alkali to prevent loss of anionogens. The ash so obtained contains salts, oxides and carbonates, and meta- and pyrophosphates not present in the original material. Organic sulfur and phosphorus appear as sulfate and phosphate. These changes occur in varying amounts with different materials, so that results cannot be compared for different tissues except for gross differences. Even with this limitation, fundamental knowledge is to be gained.

Table 2.—Ash Content of the Organs and Tissues.\*

Organ	Organ (% ash)	Ash of Organs (gms.)	Total Ash (%)
Skeleton .....	22.11	2247.3	83.1
Muscle .....	1.05	281.7	10.4
Heart .....	1.06	3.4	0.1
Brain .....	1.41	19.8	0.7
Fat tissue .....	....	....	....
Lungs .....	1.16	13.7	0.5
Liver .....	1.38	22.6	0.8
Spleen .....	1.50	2.8	0.1
Intestine .....	1.07	17.8	0.6
Kidney .....	0.80	2.4	...
Skin .....	0.70	26.9	1.0
Pancreas .....	1.05	1.0	...
Blood .....	0.85	20.4	0.7
Remainder .....	1.03	55.7	2.0
Av.	4.35†	2715.5	100.0

\* Volkmann's data of 1874 for a 62.5 kg. man. Cited by Voit.<sup>116</sup>, p. 353.

† The figure in Voit's text was 4.70.

The changes in mineral content of the body during growth are the inverse of those of water content. In the smallest fetus the amount of ash is practically negligible. It increases rapidly with growth and in the new-born infant reaches approximately 2.7 per cent, or 3.2 per cent on a fat-free basis. The ash of the human fetus was first systematically investigated by Fehling.<sup>23</sup> His data, and those of the subsequent inves-

tigations on fetuses,<sup>32, 53, 54, 78, 93</sup> on prematures<sup>9, 31, 68</sup> and on new-borns<sup>5, 12, 61, 67, 78</sup> are graphed in Figure 1.

The ash constitutes 4.35 per cent of the average adult body, or 5.2 per cent on a fat-free basis. Although the body weight of the adult is 23 times that of the infant at birth, the ash is forty times as much. Volkmann's data of 1874 will still serve to show how this ash is distributed (see Table 2). It is at once evident that the skeleton contains over 20 times the concentration of ash found in the soft parts, and that the latter organs contain about one per cent of ash. More than four-fifths of the total ash lies in the skeleton; therefore any data concerning the ash of the whole body signify primarily the content of the bones.

### MINERALS OF THE WHOLE BODY

With regard to the occurrence in the body of the various mineral elements, considerable data are at hand, and a number of reviews have been made.<sup>1, 4, 18a, 36, 45, 60a, 73, 76, 97, 121</sup> The values summarized are only a beginning of knowledge of this subject, and much more work is urgently needed in dividing total contents into their various known and unknown fractional amounts. To deal with a specific case: How much of the phosphorus occurs as organic, and how much as inorganic compounds? Is it present in the intracellular fluid, or intercellular fluid? How much is present as protein, as phospholipid, as creatine phosphate, as  $\text{HPO}_4^{--}$  and as  $\text{H}_2\text{PO}_4^-$ ? Such knowledge, covering the whole field of biochemistry, is not available at the present time. The older data on phosphorus are recorded in the monumental review by Forbes and Keith.<sup>26</sup> Similar and complete studies of the other minerals are necessary before an adequate description of the mineral composition of the body can be written.

The data on mineral composition of the whole body of the fetus at various ages and of the new-born and adult are condensed in Tables 3 and 5. Thus the only variations in body composition shown are in relation to growth. Recently it has become evident, especially from studies upon rats,<sup>39, 99, 100</sup> that there is a specific difference in the mineral composition of the bodies of males and females. This is most marked in the calcium and phosphorus. The changes in minerals of the whole body due to physiological stress, such as pregnancy and lactation, or to pathological states cannot be treated because of the lack of adequate data. The thesis of Bunge that the composition of the body resembles the milk more closely than the blood, which was modified later to state that the time required to double the birth weight is directly related to the ash of the milk, has been quoted often, but no longer excites much interest. The relation of altered composition to the food ingested, however, remains an important problem. The data on mineral composition of adult organs are given in Tables 4 and 5, but the only data available on the organs of the infant are the gross weights.

Table 3.—Mineral Content of the Whole Body at Different Ages.\*

Whole Body	Total Weight (gm.)	Fat (gm.)	Water (gm.)	Dry Weight (gm.)	Ash (gm.)	N (gm.)	Na (gm.)	K (gm.)	Ca (gm.)	Mg (gm.)	Cl (gm.)	P (gm.)	S (gm.)
Fetus, 3-4 mo. ....	126	0.6	116	10	1.5	1.0			0.42	0.022	0.34	0.27	
Fetus, 5 mo. ....	500	5	455	45	8.5	6.0	1.29	1.0	2.9	0.10	1.25	1.8	0.74
Fetus, 6 mo. ....	880	19	755	125	19.0	12	1.85	1.4	5.3	0.17	1.60	3.25	1.55
Fetus, 7 mo. ....	1155	32	975	180	30	20	2.4	2.1	6.9	0.23	2.95	4.3	1.7
Premature, 7 mo. ....	1190	36	970	220	32	20	2.8	2.1	8.6	0.25	3.05	4.4	
New-born .....	2.9	0.35	2.08	0.8	0.1	55	4.7	5.1	23.6	0.7	5.0	13.8	6.3
Adult .....	70.0	12.6	41.4	29.0	3.0	2100	63.0	150.0	1160.0	21.0	85.0	670.0	112.0
Adult/5 months fetus...	140	252	90	650	430	420	49	150	400	210	67	410	150
Adult/new-born .....	23	36	20	36	33	38	13	29	50	30	17	48	18

Table 4.—Weights of the Organs of the New-born and Adult, and Mineral Content of Adult Organs.\*

Organ	New-born		Weight		Weight		Fat		H <sub>2</sub> O		Na		K		Ca		Mg		Cl		P		S	
	(kg.)	(%)	(kg.)	(%)	(kg.)	(%)	(kg.)	(%)	(kg.)	(%)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	
Whole body	3.1	66.2	28.7	43.0	2.1	21.0	19.1	109.0	1.85	6.10	13.5	58.5	60.											
Muscles	.78	25.1	11.6	17.5	1.1	5.1	18.7	6.4	1150.00	11.0	20.	530.	16.											
Skeleton	.43	13.7	2.7	7.0	0.03	2.5	9.1	0.5	0.27	0.09	10.	0.4												
Blood serum	.13	4.2	1.8	7.3	0.7	1.2	?	7.6	?	0.11	5.2	1.8												
Blood cells	.06	1.8	4.8	19.0	8.2	3.1	6.5	4.4	0.8	0.5	12.2	2.4	18.											
Skin	.61	19.7	12.6	19.0	8.2	4.2					16.													
Subcutaneous tissue			1.4	2.2	0.17	1.1	2.1	4.1	0.15	0.2	1.8	4.6	2.9											
Brain	.38	12.3	1.8	2.7	0.38	1.1	2.7	3.1	0.17	0.31	2.3	3.2	0.5											
Liver	.14	4.6	1.4	2.2	0.13	1.1	3.0	4.5	0.21	0.12	1.0	1.5												
Intestines	.07	2.1	1.0	1.5	0.02	0.8	2.4	1.5	0.17	0.07	2.6	1.2												
Lungs	.05	1.8	0.3	0.5	0.015	0.2	0.5	0.5	0.07	0.07	0.7	0.4												
Kidney	.02	0.8	0.3	0.5	0.025	0.2	0.4	0.3	0.03	0.05	0.4													
Heart	.02	0.8	0.3	0.5	0.005	0.1			0.02	0.02	0.3	0.6												
Spleen	.01	0.3	0.16	0.2	0.005	0.1			0.02	0.02	0.2	0.3												
Pancreas	.004	0.1	0.1	0.1	0.010	0.1	0.8	0.2	0.02	0.02	0.2	0.3												

\* Calculated from the above.

\* Calculated from the literature.

Table 5.—Mineral Content per Kilogram of the Whole Body at Different Ages, and of Adult Organs, on a Fat-free Basis.\*

Whole Body	Fat (%)	Water (%)	$\overline{\text{Na}}$ (gm.)	$\overline{\text{K}}$ (gm.)	$\overline{\text{Ca}}$ (gm.)	$\overline{\text{Mg}}$ (gm.)	$\overline{\text{Cl}}$ (gm.)	$\overline{\text{P}}$ (gm.)	$\overline{\text{N}}$ (gm.)			
Fetus, 3-4 mo. ....	0.5	93			3.4	.18	15	2.7	76	2.14	69	
Fetus, 5 mo. ....	1.2	91	2.58	112	5.9	295	21	17	2.5	70	3.58	115
Fetus, 6 mo. ....	2.5	87	2.16	94	6.2	310	21	17	2.5	70	3.82	123
Fetus, 7 mo. ....	2.5	86	2.14	93	6.2	310	22	18	2.6	73	3.82	123
Premature, 7 mo. ..	3.0	85	2.42	105	7.5	375	22	18	2.7	75	3.82	123
New-born .....	12.0	80	1.78	78	9.2	460	27	23	2.0	56	5.40	174
Adult .....	18.0	72	1.09	48	20.1	1000	.36	30	1.56	42	11.6	374
Adult Organs												
Muscles .....	7.5	79	0.72	31	3.60	93	.07	4	.23	19	0.66	18
Skeleton .....	10.0	44	1.8	79	0.61	16	110.	5250	1.05	88	1.9	54
Blood serum .....	0.6	92	3.35	145	0.20	5	.11	5	.03	3	3.70	104
Blood cells .....	0.6	65	?	?	4.20	108			.06	5	1.93	54
Skin .....	15.0	73	1.6	70	1.07	27	.20	10	.14	11	3.0	85
Brain .....	12.6	90	1.7	75	3.3	85	.12	6	.16	13	1.5	42
Liver .....	21.3	79	1.9	82	2.15	55	.12	6	.22	18	1.6	45
Intestine .....	6.5	85			2.9	70	.14	7	.08	6	0.65	18
Lungs .....	1.7	78	2.5	109	1.5	39	.17	8	.07	6	2.6	73
Kidney .....	5.2	80	1.75	72	1.75	45	.20	10	.21	17	2.2	62
Heart .....	8.3	77	1.85	80	2.50	64	.10	5	.17	14	1.35	38
Spleen .....	3.0	77										
Pancreas .....	10.5	80	0.87	38	2.26	58	.17	8	.15	12	1.6	45
Thyroid .....	4.4								.19	16	1.8	51
Testicle .....	4.5								.10	8	1.8	51
Uterus .....									.10	8	2.4	67
Adrenal .....			1.45	63	1.45	37	.09	4	.10	8	2.6	74
					1.03	26	.22	11	.16	13	2.6	74
							.16	8	.10	8	2.4	67

\* Calculated from the literature.

\* Calculated from the literature.

The data in Tables 3 and 4 have been calculated in weights of the elements rather than of salts or oxides. The values in Table 3 can be easily recalculated in relation to dry material, protein or water, if desired. Table 5 shows the weights of the minerals and the meq. per kg. of fat-free substance. It would be desirable to calculate these values on a basis of the water content in terms of molal solutions, but this method can be applied only to those substances actually dissolved, and hence cannot include sulfur, calcium and phosphorus. Sodium, potassium and chlorine are discussed later in this connection (see pp. 42-46).

The calculation of the ionogen value of the various phosphorus compounds in terms of equivalents constitutes a difficult problem. In its organic combinations phosphorus may occur in non-ionized forms and the compounds in muscle are not sufficiently well defined at present to determine the proportions of the mono-, di- and trivalent ions. The equivalent value of the inorganic phosphate must be calculated as 1.8 in the blood (at pH 7.4), as 1 to 2 in the urine (at pH 4.5 to 7.8), as 2 to 3 in the feces, and as 3 in the bones. Therefore this factor must be handled as a variable to fit special problems such as mineral storage or body loss. In Table 5 the phosphorus values have been given in mM rather than meq. because of lack of specific information which would justify the latter evaluation. To calculate the sums of the positive and negative equivalents in the body (Figs. 3 and 4) the combining value has been assessed as 2.7. This computation assigns to 80 per cent of the phosphorus a value of 3.0 (the condition in the bones), and to the remainder—muscles, skin, kidneys and brain—a value of 1.5. This takes into account organic phosphorus which has no electronegative value.

These tables represent approximations based upon average analyses (and average methods). The data were collected by analysts of varying ability, working with tissues of persons dying of various diseases, and in various states of preservation, and studied for various purposes. Yet this approximation will serve for orientation of both relative and absolute amounts of the minerals in the body, and in separate organs. For specific problems it lacks both authority and accuracy.

These data will now be used to illustrate the chemical architecture of the various tissues and the distribution of the elements in the body and organs.

## MINERALS OF THE ORGANS

### Blood

So much has been written recently concerning the mineral content of blood in health and disease that no one monograph could adequately cover this phase alone. An account in Heubner's review<sup>48</sup> comprises over 50 pages. Herzfeld *et al.*<sup>46</sup> give over 22 pages of references in their account of blood calcium alone for the period 1923-28. The greater part



of Peters and Van Slyke's monograph is written from the viewpoint of alterations in the blood constituents. The purpose of this brief review of the blood (and other organs) is to discuss only so much as is necessary for an insight into their structure, with regard to inorganic elements.

The red blood cells and the plasma differ materially in their chemical composition. The specialized function of hemoglobin and its great concentration, 32 per cent of the red cells, make the blood practically what Mathews has called "the circulating tissue." The transudate of the plasma bathes all the body cells and there must be an exchange of materials between them. The exchange between the plasma and the blood cells is a special case which lends itself readily to study. When Van Slyke, Wu, and McLean<sup>115</sup> considered the blood cells and plasma in relation to their water content, a new chapter in the physiology of minerals was written. The plasma is in osmotic, ionic and acid-base equilibrium with the cells. On the water basis the blood plasma, blood cells, cerebrospinal fluid and transudates all have the same osmolar concentration. The data given in Tables 4 and 5 have been recalculated on the basis of the water content in Table 6 and in Figure 2.

Table 6.—Mineral Content of Blood and Cerebrospinal Fluid.\*

	Serum			Cells			Cerebrospinal Fluid	
	(mg./100 cc.)	(meq./l.)	(meq./l. H <sub>2</sub> O)	(mg./100 cc.)	(meq./l.)	(meq./l. H <sub>2</sub> O)	(meq./l.)	
Na .....	330	143	154	23	10	15	151	
K .....	20	5	5.3	420	105	163	4	
Ca .....	10	5	5.1	0	0	0	3	
Mg .....	2.7	2.2	2.4	6.6	5.5	8.5	5	
Total cations .....		155.2	166.8		120.5	186.5	163	
PO <sub>4</sub> as P .....	3-6	1-2	2	0	0	0	1	
SO <sub>4</sub> as S .....	1	1	1	?	?	?	1	
						Oxygenated	Reduced	
Cl .....	365	103	111			80	84	125
	(vol. %)							
CO <sub>2</sub> .....	60	27	29			20	30	28
Proteinate .....		17	19			65	50	
Total anions .....		150	162			165	164	155

\* Values for blood from the literature; for cerebrospinal fluid from Pincus and Kramer<sup>80</sup> and Hamilton.<sup>81</sup>

The blood plasma closely resembles the other body fluids—transudates, exudates and secretions. Such minor differences as exist are attributable to the varying protein content, from cerebrospinal fluid which is protein-free, to edema fluid, with 0.1 to 0.2 per cent; ascitic fluid, 0.8-4.6; pleural effusions, 1.0-3.3; tuberculous pleural effusions, 3.4-5.6;

empyema pus, 5.6;<sup>35, 38</sup> and blood serum, 6.0-7.5 per cent. Proteins are related to mineral distribution in a number of ways. The proteins function as anions on the alkaline side of their isoelectric point. Albumin and globulin differ in their degree of ionization, but the specific differences of the other proteins are not well known.<sup>15</sup> Proteins also diminish the water content, because of their bulk.

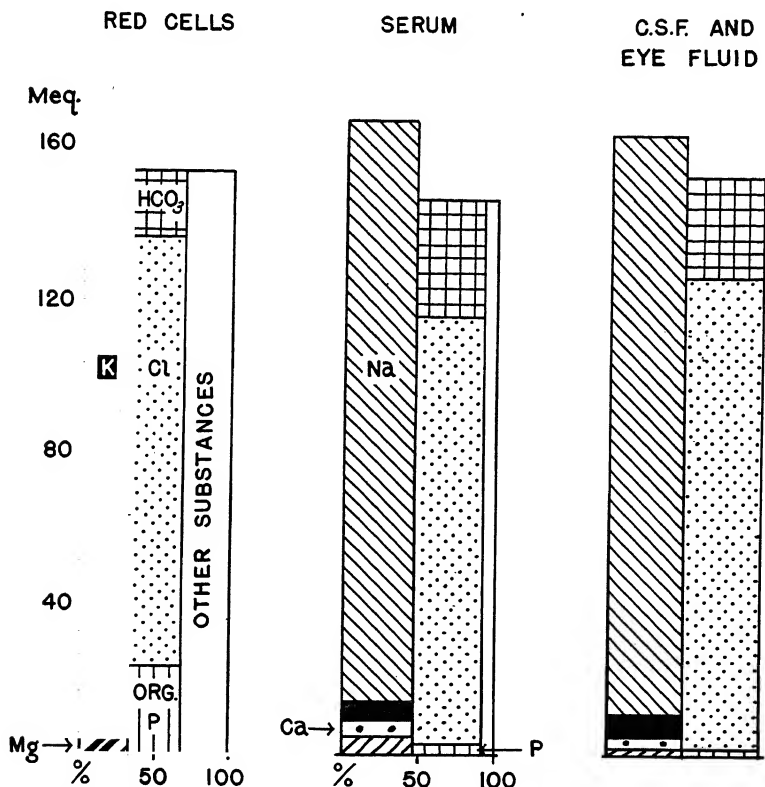


FIGURE 2. Mineral Content of Body Fluids per Kilogram of Water.\*

\*From data in Table 6.

The gross composition of the plasma with respect to inorganic constituents may be compared with physiological saline (0.9 per cent NaCl), which contains 154 meq. of anions and of cations per liter. The main mineral constituents are sodium and chloride. The chloride content is about 100 meq./l. of plasma (0.6 per cent NaCl) and the sodium 143 meq./l. (0.84 per cent NaCl). See Table 6.

Red blood cells (and other cells) differ markedly from plasma, the main cation of the former being potassium and of the latter sodium. The cells and plasma cannot exchange cations, but anions must be transferred if electroneutrality is to be maintained during the changes which are discussed later. The study of the role of hemoglobin in this physico-chemical system has been exhaustively reviewed.<sup>44, 86, 88b, 113</sup> When the oxygen of oxyhemoglobin in blood is released and reduced hemoglobin is formed with a slight increase in pH, chloride passes from the serum to the cells, bicarbonate increases in both, and water is transferred from the serum to the cells. Chloride in the serum water changes from 113 to 103 meq., in the cells, from 82 to 87; and the bicarbonate changes from 24 to 34 in serum and from 17 to 31 in cells.<sup>88c</sup> Oxygenation alters the strength of the hemoglobin as an acid so that it dissociates more. Oxygenated hemoglobin therefore neutralizes more cations and hence the cell chloride diminishes. Acidification with CO<sub>2</sub><sup>88d</sup> increases the bicarbonate and chloride within the cells and also the intracellular water.

Under conditions in which the bicarbonate is increased, the chloride is usually decreased, and *vice versa*. However, although the individual anions may change markedly in pathological conditions or even with physiological variations, the cations of the cells and serum are altered only slightly even *in extremis*.

**Electroneutrality.**—The summation of the analytical values for the anions and cations in the serum water (Table 6) shows essential equality at 167 meq. of cations and 162 of anions. The agreement is within the experimental error, even without allowing for a small amount of lipid-chlorine which Peters has reported.<sup>87</sup>

The data for the cells are, however, far from satisfactory. They show a preponderance of cations, which is not in agreement with the recent calculations of Peters.<sup>86</sup> He gives a lower value to the potassium in the cells and greater water content, so that the total cation value per kg. of cell water becomes 144 meq. compared to the value we have calculated as 186 meq. He has further recalculated the anions as 189 meq., compared to our value of 165 meq. This difference occurs principally through his assigning to the organic phosphorus an ionic value of 1.0 eq., though this is probably too small, whereas we have, through lack of convincing evidence, given it a value of 0. Our values give an excess of cations of 22 meq. (which is exactly the value he has assigned to phosphorus) and his values give an excess of 43 meq. of anions. Peters calculates that the total cation concentration of cell water is less than that in serum water, whereas our calculations show the opposite. It has always been considered a strong argument that, at the acidity of the blood, more cations should be present in the fluid which contains the greater amount of proteinate. Peters' argument that anions and cations

may be combined with protein as B-protein-A salts is in accord with the known capacity of hemoglobin to unite with  $\text{CO}_2$  to form carbhemo-globin. This type of combination would perhaps apply to the other anions also, although this reaction has not been proved for the blood cells. Thus some anions in excess of cations might be present without upsetting the demands of electroneutrality.

From the distribution ratios between cells and serum of  $\text{H}^+$  (0.5/1),  $\text{HCO}_3^-$  (0.8/1) and  $\text{Cl}^-$  (0.65/1),<sup>114</sup> Peters calculates that, at a pH of 7.1 within the cell, the  $[\text{HCO}_3^-]$  is reduced 13 meq. and the  $[\text{Cl}^-]$  21 meq. If these 34 meq. are subtracted, the excess of anions is greatly reduced, and the cells, as the serum, are calculated to be in a state of electro-neutrality.

**Osmotic equilibrium.**—That the minerals within the cells and in the interstitial fluid are in osmotic equilibrium can be demonstrated better in the blood than in any other tissue. The simplest method of measuring this pressure is by freezing point determinations. The depression of the freezing point,  $\Delta$ , equals  $-1.86^\circ\text{C}$ . per mol of dissolved substance. When dealing with electrolytes the term *osmolar*, or *osmotic equivalent*, is convenient. For example, if a substance splits into two ions the osmolar value is twice the molar value. In normal blood serum  $\Delta = -0.56^\circ\text{C}$ . (0.50 – 0.60). This value may be converted to milliosmols as follows:

$\frac{0.56}{1.86} \times 1000 = 302 \text{ mosM/l. of serum (milliosmolar)}$ . On the basis of

water contained this is  $\frac{302}{92} = 330 \text{ mosM/kg. of water (milliosmolal)}$ .

The total osmotic pressure is equal to the sum of the partial pressures, *i. e.*, the effects of the individual ions or molecules on the activity of water are additive. Of this total about 12-14 mM are present as urea and dextrose. The proteins which, when considered as anions, represent about 17 meq., owing to their large molecular weights have small osmotic pressure, about 2 mM. When the values for dextrose, urea and protein are subtracted from the total, about 315 mosM remain to be accounted for by the electrolytes.<sup>104a</sup> Normally the other substances known and unknown represent a very small fraction. The electrolytes dissociate to different degrees or show different degrees of activity. But the predominant ions are  $\text{Na}^+$  and  $\text{Cl}^-$  which, because of their complete ionization, give a depression of the freezing point nearly twice that of the molar concentration calculated for sodium chloride.

As a first approximation of the calculated osmotic pressure from chemical analysis we may take twice the total cation concentration, or the sum of the cations and anions. The osmotic pressure calculated from twice the cation value (see Table 6) is  $(167 \times 2) - 3 = 331 \text{ mosM/kg. of water}$ , and from the cations plus anions,  $(167 - 3)$

+ (162-17) = 309 mosM. (The 3 subtracted in the first case represents the difference between the combining value and the molal value of the calcium and magnesium, and the 17 is the corresponding value of the proteins). Of the two methods of computation, obviously the second is preferable theoretically, and gives values closer to those observed by the freezing point method. Both show rough agreement between the calculated and observed values of osmotic pressure. However, the uncertainty of the data makes the values thus computed, even for serum, serve only for orientation or approximation.

As in the case of electroneutrality, the computation of the osmolar value of the cells is much more uncertain than for serum. Here the proteins occur in such large proportion that they must be specially dealt with. The calculations made by Peters are of interest. He has (1) added to the anions the 23 mM of organic phosphorus, (2) calculated the osmotic pressure of hemoglobin as 7 mM against its combining value of 64 meq., (3) subtracted the anions combined with protein,  $\text{Cl} = 17$  and  $\text{CO}_2 = 13$  mM, and (4) calculated that the hemoglobinate is ionized only to the extent of 50 per cent. The value so obtained, 210 mosM/l., is however so low that it indicates merely an honest acknowledgment of the lack of adequate data, for the cells must be in osmotic equilibrium with the serum.

**Individual minerals.—Sodium and potassium.**—The data given for  $[\text{Na}^+]$  and  $[\text{K}^+]$  in Table 6 represent the values usually assigned to human blood. But the sharp differentiation of  $\text{Na}^+$  in serum and  $\text{K}^+$  in cells found in man and the primates differs from that in certain other species. In the dog and cat, for example, the  $[\text{K}^+]$  in the cells may be as little as the  $[\text{Na}^+]$  in human cells, of the order of 5-10 meq.<sup>58</sup> But the  $[\text{Na}^+]$  in these species is so high that the sum of  $[\text{Na}^+]$  and  $[\text{K}^+]$  in all species is practically constant, and represents about 110 meq./l. of cells. These variations in  $[\text{Na}^+]$  and  $[\text{K}^+]$  are accompanied by differences in phosphorus compounds, but have not been correlated with either the whole or any fraction of the phosphorus value.

**The calcium** in the blood occurs wholly or almost wholly in the serum. Although small in amount,  $10.5 \pm 0.8$  mg./100 cc. of serum (5.25 meq./l.) and  $5.3 \pm 0.3$  mg. (2.65 meq.) in cerebrospinal fluid, the calcium is of great physiological importance in maintaining the normal neuromuscular irritability and bone structure. With regard to the state of calcium and phosphorus in the blood and their significance, more detailed accounts will be found in Chapters 6 and 7.

**The magnesium** of the blood, unlike the calcium, is not confined to the plasma. It is present in the cells in even greater concentration than in the serum. The values for whole blood, cells and serum are, respectively: 4.6, 6.6 and 2.7 mg./100 cc.; this equals, in meq./l., 5.5 for cells and 2.2 for plasma, and on the water basis these values become 8.5 and

2.4 meq./kg. of water. The values vary within narrow limits in health and disease.<sup>34</sup> The function of the magnesium in both cells and body fluids is still obscure in many respects. (See further under *Magnesium*.)

*Ammonium* in blood serum has been variously reported as being absent, or present in traces of not more than 0.05 per cent. (See further in Chapter 5.)

*The phosphorus compounds* of the blood can best be divided into phospholipids and acid-soluble phosphorus, and the latter subdivided into inorganic, ester and nucleotide phosphorus. The main difference between the serum of infants and adults lies in the greater inorganic phosphate in the serum of infants,  $5.0 \pm 1$  mg./100 cc. (1.6 mM/l.) compared with  $3.0 \pm 1$  (1.0 mM) in the adult. This value varies with diet, season and vitamin D intake. The inorganic phosphate content of cells is small, and its presence *in vivo* has been questioned. After removal from the body this component increases and may be transferred to the serum. The various organic forms of phosphorus and sulfur are discussed in chapters on *Phosphorus* and *Sulfur*.

*Chloride*.—The  $\text{Cl}^-$  in the blood serum is quantitatively the most prominent of the anions; the 103 meq./l. form  $\frac{2}{3}$  of the anions (see Table 6). The  $\text{Cl}^-$  plus the  $\text{HCO}_3^-$  and proteins (functioning as anions on the alkaline side of their isoelectric point) account for practically the whole anion value except when  $\beta$ -hydroxybutyrate or some other anion is abnormally present. Chlorolipids to the extent of 4 meq. have been reported.<sup>87</sup> The relation between the chloride in cells and that in serum have been investigated in detail.<sup>33, 114, 125</sup> Ordinarily the cells, per unit volume, contain only 45-55 per cent of that in the plasma. When considered on the water basis, because of the high protein content of the cells, the difference is not so marked, about 75-80 per cent of that in plasma water. Thus whole blood chloride determinations indicate the proportion of cells in blood. The  $[\text{Cl}^-]$  is governed by that of water and  $\text{HCO}_3^-$ , by the acid-base equilibrium, the state of oxygenation and reduction and the Donnan equilibrium. The fundamental facts are that  $\text{Cl}^-$  is distributed in all fluids of the body and that red blood cells are probably unique among cells in their high  $[\text{Cl}^-]$ . The greater the protein content the less the  $[\text{Cl}^-]$ . When changes in the acid-base equilibrium occur, the  $\text{Cl}^-$  (but not the cations) can pass either in or out of the red cells and together with the water is distributed according to the requirements of ionic and osmotic equilibria. See also *Mechanism of Acid-Base Equilibrium*, in Chapter 13.

The  $[\text{Cl}^-]$  is greater in blood cells than in muscle cells, the concentration in the plasma is less than in edema fluid and transudates (110-115 meq./l.); it is maximal in the protein-free cerebrospinal fluid (125 meq.).

*Bicarbonate*.— $\text{HCO}_3^-$  is not only one of the principal anions in the blood, but because of special chemical properties, it is of major impor-

tance in the maintenance of the acid-base equilibrium in the body fluids. Normally it is present in plasma to the extent of 27 meq. of  $\text{HCO}_3^-$ /l., which when liberated as  $\text{CO}_2$  is equal in volume to 60 per cent of the serum (called 60 vol. per cent). Because of their greater protein and lesser water content the cells contain less  $\text{HCO}_3^-$  than the serum, which in turn contains less than transudates; cerebrospinal fluid, which is protein-free, contains most. The average  $[\text{HCO}_3^-]$  of the red blood cells is about 15 meq./l. When this concentration is calculated on the basis of the water content, it is about 22 meq./kg. of water. The  $[\text{HCO}_3^-]$  is governed by the same forces mentioned above, which influence the  $[\text{Cl}^-]$ . Oxygenation lowers the  $[\text{HCO}_3^-]$  of the cells and reduction increases it; see Table 6.

The  $\text{HCO}_3^-$  is especially important in acid-base equilibrium. It has a unique capacity to buffer the blood at its normal acidity of about pH 7.40. This is dependent upon its ability to combine with  $\text{H}^+$  to form gaseous  $\text{CO}_2$ , which is readily diffused and excreted by the lungs; upon the capacity of the respiratory center to alter the  $[\text{H}_2\text{CO}_3]$  in the blood; and upon its special relations to hemoglobin, which permit the acceptance of  $\text{HCO}_3^-$  formed in metabolism and the simultaneous yielding of oxygen without marked change in the pH of the blood. The  $\text{HCO}_3^-$  is in equilibrium with the other buffers of the blood and therefore may be used as an indicator of the state of the acid-base equilibrium of the whole system. The pH of the blood is determined by the relation of the  $\text{CO}_2$  tension—and hence of the  $[\text{dissolved CO}_2]$ ,  $[\text{free CO}_2]$ , and  $[\text{H}_2\text{CO}_3]$ —to the  $[\text{HCO}_3^-]$ . This relation is shown in the Henderson-Hasselbalch equation, in quantitative form. A description of this mechanism is given in Chapter 13.

### Cerebrospinal Fluid

Cerebrospinal fluid is probably a secretion, but closely resembles a dialysate of blood plasma.<sup>77</sup> Its composition approximates that which would be postulated by the Donnan equilibrium theory for a fluid which contains no protein.<sup>27</sup> However, as has been frequently pointed out, the body neither provides ideal semipermeable membranes nor acts exactly in accordance with known laws. The data are shown in Table 6 and Figure 2.<sup>27, 59, 119</sup> The  $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$  are greater than in blood serum (for protein functions as an anion in the serum). The cations, especially  $\text{Mg}^{++}$ , are present in too great a concentration to be accounted for by the above theory. The data show a small anion deficit. If this is real and not due to either analytical error or physiological variations, some other anion must be present, for the pH of the cerebrospinal fluid is the same as that of the blood. (The slight divergence predicted by the Donnan equilibrium theory is within experimental error.) The cerebrospinal fluid system is isolated and equilibrium with the blood serum is

attained slowly, if at all. Local infection, as in meningitis, and possibly encephalitis and epilepsy, may produce conditions in which the pH of the cerebrospinal fluid is definitely more acid than that of the plasma.

The calcium of the cerebrospinal fluid forms an interesting special case of  $[Ca^{++}]$  in body fluids. In the blood serum approximately one-half of the calcium is present as calcium proteinate and remains non-ionized. In the cerebrospinal fluid there is almost no protein; therefore total calcium and ionized calcium are practically identical. Conditions which affect the total calcium in the blood are not reflected proportionally in the cerebrospinal fluid. Tetany, which may be associated with a 50-per cent lowering of the blood calcium, involves only a 20-per cent lowering of that in cerebrospinal fluid. Similarly, injections of calcium salts and parathyroid hormone do not raise the concentration of calcium in the cerebrospinal fluid proportionally to that in the blood and other protein-containing body fluids.

*Other body fluids*, aqueous and vitreous humor, hydrocele fluid, joint fluid, pleural fluid, lymph,<sup>21</sup> peritoneal fluid, and also ascitic and edema fluids, closely resemble dialysates of blood serum. The calcium concentration is lower than in serum and is proportional to the protein concentration; the chloride is higher. Amniotic fluid, although a dialysate plus the excretory products of the infant, is unique in that, with a protein content of 0.2 per cent, it has a calcium value of 8.0 mg./100 cc. How so much calcium is held in solution is unknown.<sup>13, 74, 102</sup>

## Muscles

The muscles of the body in adult life comprise 41 per cent of the body weight, and contain 55 per cent of the body water, but only 10 per cent of the ash. On a fat-free basis, muscles contain about 75 per cent of water and 20 per cent of protein. The calcium, magnesium, chlorine and iron contents of the ash of the adult have been given by Magnus-Levy.<sup>73</sup> For further knowledge we are dependent upon the analyses of Katz<sup>56</sup> made in 1896.<sup>20</sup> In the table below are recorded not only the values per 100 gm. of fresh muscle, but also the values which Hill and Kupalov<sup>50</sup> have calculated on the basis of the contained water. (See also Tables 4 and 5 and Figure 4.)

Mineral Content of Muscle.

	Unit	Na	K	Ca	Mg	P	Cl	CO <sub>2</sub>	Lactic Acid
Katz	mg./100 gm. fresh muscle	72	365	10	27		60		
Hill and Kupalov	meq./kg. of water	44	132	3	16	78	23	16	2

This information forms the basis of the well-known generalization that the cells are the major storehouse of potassium in the body. This



information is not new; it was known for half a century before Katz. Little has been added since, although newer studies are endeavoring to relate the potassium content to muscle physiology.<sup>17, 123</sup> The evidence that muscle potassium is in a "bound" form is not convincing.<sup>11</sup>

The low chloride content of muscle in comparison to that of blood serum indicates that other substances furnish the main portion of the anions in muscle. Of the total chloride in muscle tissue Hill calculated that probably not more than 82 per cent is within the cells. Recent studies<sup>24, 41, 42</sup> tend to show that all the chloride is extracellular. The  $[Cl^-]$  in muscle is one-fifth that in the blood plasma. If all the chloride is in the intercellular water, and if it is in the same concentration as in blood plasma, the intercellular water represents only one-fifth of the muscle water. Hence four-fifths of the water is intracellular and one-fifth is in the spaces between the cells. Muscle solids constitute 25 per cent of the total weight; therefore the interstitial water becomes 20 per cent of the remaining 75 per cent, or 15 per cent of the total muscle, and the intracellular water 60 per cent of the total muscle.

The muscle cell in life seems impervious not only to cations as in the case of the red blood cells, but also to the anions  $Cl^-$ ,  $H_2PO_4^-$ ,  $HPO_4^{--}$  and  $SO_4^{--}$ . Alterations in minerals in the interstitial fluids and changes in the acid-base equilibrium bring about an altered condition within the cell by exchange of water. In this way only is osmotic equilibrium established. If the intercellular water is increased or decreased and is isotonic, the muscle cells remain of constant size, but the whole muscle must swell or shrink. If the interstitial fluid is hypo- or hypertonic, the muscle cells must swell or shrink respectively. The volume of the whole muscle depends upon the sum of the two compartments of muscle water.

In 1907 Fletcher and Hopkins first made satisfactory measurements of the lactic acid in muscle, and this began the modern era of muscle physiology. Embden, Meyerhof, Hill and many others have competed and collaborated to the benefit of all. Outside of the heat changes involved, the investigations have centered about the origin of lactic acid and its reversible reaction. It has become evident that the carbohydrates which yield lactic acid are dependent for their physiological action upon phosphorus compounds. Embden suggested the term "lactacidogen" (which he first considered a hexosediphosphate and later a hexosemonophosphate) for the material determined as phosphate, produced when a muscle hash is incubated with bicarbonate. In 1927 Eggleton and Eggleton<sup>22</sup> showed that muscle contains a substance which they called "phosphagen," a labile organic form of phosphorus. Simultaneously and independently Fiske and SubbaRow<sup>25</sup> in 1927 and 1929 proved that what for many years had been regarded as inorganic phosphate is in reality a labile organic compound containing phosphoric acid and creatine in equimolecular proportions. Further, they reported that

practically all the labile phosphorus in the protein-free filtrate of muscle is in the form of phospho-creatine. Muscle tissue contains about 75 mg. of phosphorus as creatine phosphate, per 100 gm. Lohmann<sup>71</sup> found another substance which had the properties of lactacidogen. When isolated, he found it to be adenosinetriphosphate. Hill<sup>49</sup> has summarized these findings under the title, "Revolution in Muscle Physiology." The interactions between these different phosphate systems and carbohydrate systems form a long chain of events, a simplified account of which is given in Chapter 7, *Phosphorus*.

The iron content of the muscle cell is small but presumably important. Macallum<sup>72</sup> identified it with the chromatin of the nucleus, and thus indicated its primordial and fundamental relation to all cell life. Recent studies have related it to the respiratory functions of the cell.<sup>118</sup>

The analysis of heart muscle and of the uterus, and of bovine smooth muscle compared to that of striated muscle, shows about twice as great  $[Na^+]$  and  $[Cl^-]$  and a lower  $[K^+]$ , but the calcium and magnesium vary little in different types of muscle. No marked deviations in the calcium content of muscle have been observed in experimental or pathological conditions. Exceptions are the increase after dosage with irradiated ergosterol and decrease of 40 per cent in ricketic rats.<sup>48</sup> The significance of any finer changes is obscured, not only by differences in methods but also by the variation found from case to case and between muscles from the two sides of the same individual. For the present, the gross differences must be attributed to varying amounts of intercellular fluid.

### Skeleton

The last decade has enjoyed a renaissance in the study not only of muscle but also of bone. Investigations of experimental rickets and vitamin D rendered it necessary, if there was to be a fundamental advance in the knowledge of the physical chemistry of calcification, that the structure of bone salts be better understood, for the form of the chemical substrate determines the equilibria involved in the precipitation of salts in bone formation. Analyses of bone ash show that approximately 50 per cent of the ash is calcium, of which 85 per cent is in the form of  $Ca_3(PO_4)_2$  and 12 per cent of  $CaCO_3$ . Magnesium constitutes 0.5 per cent of the ash; sodium, potassium and chlorine are frequently neglected because they are present in such small amounts.

Howland, Marriott and Kramer<sup>51</sup> showed that, after excluding the  $CaCO_3$ , the Ca/P ratio in bone is the same as in  $Ca_3(PO_4)_2$ . But there is considerable doubt as to the existence of tertiary calcium phosphate, as such. Long ago Gabriel<sup>28</sup> felt that some water must be included as water of constitution, as calcium hydrate in combination with calcium phosphate, and there is other evidence now available to show that the salts of bone are more than simple mixtures of  $CaCO_3$  and  $Ca_3(PO_4)_2$ . X-ray

analyses<sup>7, 14, 55, 90, 105</sup> and chemical analyses<sup>6, 30</sup> have shown that bone probably belongs to the apatite series of minerals,  $n\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$ , in which F, OH, etc. may replace  $\text{CO}_3$ , and the value of  $n$  lies between 2 and 3. Among the numerous possibilities bone salts most closely resemble dahllite.<sup>90</sup> Morgulis<sup>80</sup> has made chemical analyses of the "glycerine ash" of bones of various species, and found the composition to be:  $\text{Ca}_3(\text{PO}_4)_2$ , 77.20 per cent;  $\text{CaCO}_3$ , 11.81 per cent;  $\text{Ca}(\text{OH})_2$ , 3.13 per cent. The molar relationships between  $\text{CaCO}_3$  and  $\text{Ca}_3(\text{PO}_4)_2$  do not suggest  $\text{Ca}[\{\text{Ca}_3(\text{PO}_4)_2\}_3]\text{CO}_3$ , but correspond closely to  $\text{Ca}[\{\text{Ca}_3(\text{PO}_4)_2\}_6](\text{OH})_2$ .<sup>59a</sup> That the salts are not precipitated as found in bones, but are first deposited as  $\text{CaHPO}_4$ , and later altered, was suggested by Shear and Kramer<sup>96</sup> and Klement.<sup>59b</sup> There are a number of recent reviews on the composition of bone.<sup>48, 52, 57, 60b, 88e, 110</sup> The main trend of modern opinion follows the thesis of Hoppe, 1862, that the arrangement of inorganic elements in bone is similar to that in the apatite minerals.

The average composition of the skeleton is given in Tables 4 and 5 and Figures 3 and 4. The amount of total minerals in bone is variable, but little variation is shown in the ratio of Ca/P. Regardless of the form of the compound, this proportion is so fixed that wherever bone is laid down or bone salts removed the ratio of Ca/P remains practically unaltered at 2.15. For this reason the per cent of total ash of the bones measures the amount of the individual minerals laid down in the organic matrix just as well as the per cent of calcium and phosphorus. The percentage of ash at various ages in the human being is known only approximately, but has been especially well studied in the rat.<sup>39, 98</sup> The lime salts in the bones are laid down only in the latter part of fetal life, and increase day by day until in the adult rat the ash is 39 per cent of the fresh bone, or 62 per cent of the dried extracted bone, and hence is greater in amount than either water or organic material.

The carbonate content of the skeleton may readily be calculated. If 12 per cent of the bone ash is  $\text{CaCO}_3$ , this equals 370 gm. or 7.4 eq. in the whole body or 630 meq./kg. of fresh bone. That the carbonate-to-phosphate ratio should show pathological variation seems probable, as it is well known that the bones of youth differ from those of old age in that the latter are more brittle and contain more carbonate.<sup>83</sup> This same variation in the ratio of bicarbonate to phosphate is present in the blood in infancy and old age.

The magnesium of bone forms a fairly constant fraction. For most purposes it has been assumed to be magnesium phosphate, although magnesium carbonate has also been found. Whether it forms an integral part of the complex mineral substrate has never been demonstrated. In rickets, decrease of calcium content has been found associated with

increased magnesium and forms one of the many examples of the inverse relationship of the alkaline earths. (See Table 13, p. 148.)

When bone is considered in the fresh state (and not as extracted ash) the large amount of body fluid therein contains a considerable portion of the body sodium and chloride. With increasing age the percentage of fluid diminishes and therefore the fraction of these salts in bone is also decreased. It has been known since the time of Bunge that cartilage is rich in sodium, about 0.5-0.6 per cent. The amount of cartilage decreases with age, and with it this fraction of sodium also.

Further variability in composition is shown by the occasional presence of lead, beryllium, strontium, radium and fluorine, depending upon the amount ingested. During periods of stress the stores of minerals in the skeleton are depleted, or during abundance, built up, especially in the trabeculae. Therefore, neither chemically nor physiologically is the skeleton an organ of fixed and invariant composition whose only purpose is to serve as a framework upon which the muscles can act to mechanical advantage. It resists alteration in its relative composition as do other body structures. It constitutes the main repository of calcium, phosphorus and carbonate, and to an unexpected extent also of magnesium, sodium and potassium. Through its ability to store or release all of the minerals which it contains, it is of fundamental importance in the transfers of minerals in the body.

The special problems concerned with the relation of bone to parathyroid hormone, vitamin D and phosphatase are discussed under these headings in Chapters 4, 6 and 14.

## Skin

The integument and underlying connective tissue, with the contained fat, may be greater in weight than the skeleton, and second only to the muscles, and hence there is some foundation for the phrase "skin and bones." Structurally this tissue differs markedly from the other two. (See Tables 4 and 5, and Figure 4.) The study of the minerals of the skin<sup>8, 82</sup> reveals that a considerable amount of sodium and potassium are stored here, and a minimum of calcium, magnesium and phosphorus. Studies on dogs<sup>85, 117</sup> and infants,<sup>61, 62, 91</sup> and more recently on adults,<sup>112</sup> emphasize that this tissue is the normal site of chloride storage. The state of edema shows the capacity for the extension of this storage, but it is not widely appreciated how much extracellular fluid is found here under normal conditions. About 30 gm. of chloride, equivalent to 5.5 l. of 0.9-per cent saline solution are present here. According to this calculation about one-third of the body chloride is in the skin and subcutaneous tissue. The chloride content of the skin is not only large but variable, 0.24-0.48 per cent. The skin contains also considerable silicon (50 mg./kg.). This alone of the elements decreases with age; this fact

fits well with the observation<sup>66</sup> that the elasticity of the skin is proportional to the silicon content.

### Nerve Tissue

Phosphorus is unusually abundant in nerve tissue. We still owe most of our knowledge of the brain and nervous tissue to Thudichum<sup>111</sup> and W. Koch.<sup>63</sup> Both of these men were primarily interested in the complicated organic structure, and made separations of the phospholipids and sulfolipids. The composition of nervous tissue has been summarized.<sup>57a, 94, 120, 124</sup> (See Tables 4 and 5, and Figure 4.) The gray and white matter differ materially; the former contains 84 per cent water, and the latter 69 per cent. Only a portion of the water-soluble phosphorus and sulfur compounds are inorganic. Koch found, in the gray and white matter respectively, that the inorganic fraction constituted 5.9 and 3.4 per cent of the total sulfur, and 13.0 and 7.0 per cent of the total phosphorus. Variation in chemical structure occurs also in the cerebellum, spinal cord and nerves. The proportion of phosphorus is largest in the spinal cord, 0.548 per cent; next in white matter, and least in the gray, 0.239 per cent.<sup>120</sup> Calcium and magnesium are similarly distributed. The sodium, potassium and chloride are approximately the same throughout. The data in the literature concerning absolute amounts of cations are especially conflicting.<sup>48</sup> The ratio of K/Ca is unusually high. Most of the organs contain more sodium than potassium; brain, muscles and liver are exceptions.

The water in the nervous substance is held with great tenacity, even when the rest of the body is dehydrated.<sup>109</sup> The water content of the brain diminishes with age<sup>64</sup> as does the phosphorus; and although this finding has been criticized it is probably correct.<sup>70</sup>

The Mg/Ca ratio has also been shown to decrease with age,<sup>19</sup> for the calcium may increase. It has been reported that, with diets of low calcium-high phosphorus content, or in rickets, the calcium content of the brain was diminished, but returned to normal after cure.<sup>47</sup>

Iron, presumably not from blood, and traces of manganese, zinc, copper, bromine and arsenic occur in normal brain tissue. Further details of the chemical structure of the brain are found in Chapter 7, *Phosphorus* and Chapter 8, *Sulfur*.

### Other Organs

Mineral analyses of the remaining organs are fragmentary and unsatisfactory because undoubtedly some of the minerals given in the analyses are due to the included blood cells and serum, and an unknown amount of intercellular fluid; see Tables 4 and 5. Data are available for the spleen, lungs, kidney, intestines, salivary glands, the thyroid and testicle,<sup>73</sup> uterus,<sup>65, 127</sup> lymph glands,<sup>106</sup> adrenals and pancreas.<sup>75</sup> New

growths have no distinguishing mineral content.<sup>95</sup> The minerals of the thyroid gland<sup>79</sup> are not remarkable except for the iodine content, which naturally has been the center of interest, and is discussed in Chapter 10, *Iodine*. The pituitary gland has recently been found to be rich in bromine. Most organs contain about 1 mg./100 gm., but here the bromine content is 15-30 times as large—highest in the anterior lobe, next in the pars intermedia, and least in the posterior lobe.<sup>126</sup> The elements which occur in traces only have recently assumed a new importance in the structure of organs, and are discussed in Chapter 11. Still to be proved is the meaning of the chemical anatomy of these organs in relation to function.

### Quantitative Distribution

When considered *per kg. of fat-free substance*, as in Table 5, it is obvious that the organs differ materially in mineral composition. *Sodium* (except in blood) varies from 30 to 100 meq. The skeleton, because of its large store of sodium in cartilage and the high extracellular fluid content is surprisingly rich in sodium.<sup>10, 103</sup> *Potassium* is stored in cells, especially in muscle and red blood cells. There is least in blood serum, skeleton, skin and lungs. Muscles differ materially in  $[K^+]$ ; skeletal muscles contain one-half more than the heart and four times more than the uterus. *Calcium*. Apart from the skeleton, the organs vary in calcium concentration from 3.5 meq. in muscle to 10 meq. in skin and kidney. *Magnesium* exhibits a similar variation, but is of about twice the concentration in all the organs except the skeleton. *Phosphorus* in the organs shows a wider range of variation than calcium and magnesium—18-122 mM, excluding blood serum and skeleton—as well as a higher concentration, roughly 25 times the calcium (on the molar basis). This is commensurate with the multiplicity of phosphorus compounds and their functions. *Chlorine* concentrations, although lower, roughly parallel those of sodium. This is the only element in which the fractions indicated in Table 4 do not add up to a total roughly equal to that of the whole body. This discrepancy is only partially accounted for by the cerebrospinal fluid, which, although rich in chloride, is small in amount, and cannot account for more than 0.5 gm. The large missing fraction must be sought in the subcutaneous tissue which, together with the skin, forms the elastic depository for this element. Question has arisen whether the chlorine may not exist in organic forms, but to date the inorganic chloride must be considered as the sole important state of chlorine.<sup>87</sup>

In summary it is obvious that the skeleton is the main mineral storehouse for calcium, phosphorus and carbonate, and that it also contains one-half of the body magnesium and one-third of the sodium. The muscles form the second great reservoir with three-fourths of the potas-

sium, and one-third of the magnesium and sodium. The chlorine is so evenly distributed among the organs that none is its chief site. The blood serum, which we think of as the depository of sodium and chloride, contains only half the amount of sodium and chloride found in the muscles or skeleton, and the red corpuscles, which we think of as potassium-rich, contain hardly more than do the potassium-poor bones.

#### RELATION BETWEEN POSITIVE AND NEGATIVE MINERALS IN BODY STRUCTURE

In order that life may continue, the neutrality of the body must be maintained. The mechanism requires equality of anions and cations occurring in various buffer systems—carbonates, phosphates and proteinate. A great deal has been written of the acid-base equilibrium of the blood (see p. 274), but that of the body tissues remains an uncharted field. The circulating fluid is in approximate equilibrium with the tissues, but these are separated by membranes, or systems which act like membranes, of varying and selective permeability. Further, the components occur in different phases. The exact carbonate content of the tissues remains unknown, and also the buffering capacity of the various phosphorus and protein fractions. Until the pH within the cells is better known than at present, an accurate analysis is not possible. However, it seems desirable to indicate roughly the relation between positive and negative ionogens in the structure of the body and organs. The following summary is calculated from the data given in Table 3, for the whole body of the adult, and is expressed in equivalents. (See also Figure 3.)

Positive eq.	Na	2.8	K	3.3	Ca	57.5	Mg	1.7	Total	65.3
Negative eq.	P	58.0	Cl	2.4	S	—	HCO <sub>3</sub>	—	Total	60.4
										<hr/>
										Excess positive eq. 4.9

It is at once obvious that the calcium represents over 88 per cent of the positive equivalents in the body, and the phosphorus a similar proportion of the total negative equivalents. Because the great preponderance of phosphorus occurs in bone it seems improbable that the assumptions made regarding the ionogen value of phosphorus should lead to an error of more than 1 or 2 eq. The proteinate, sulfate, carbonate, bicarbonate and unknown anions not included in the summary account for only 8 per cent of the total negative eq., if electroneutrality is assumed. The carbonate of bone ash neutralizes about 13 per cent of the positive eq. in bone. This carbonate alone, 7.4 eq., added to the negative eq. would cause them to be in excess of the positive eq. To this should be added the bicarbonate in the rest of the body. The bicarbonate neutralizes about 16 per cent of the total cations in serum.

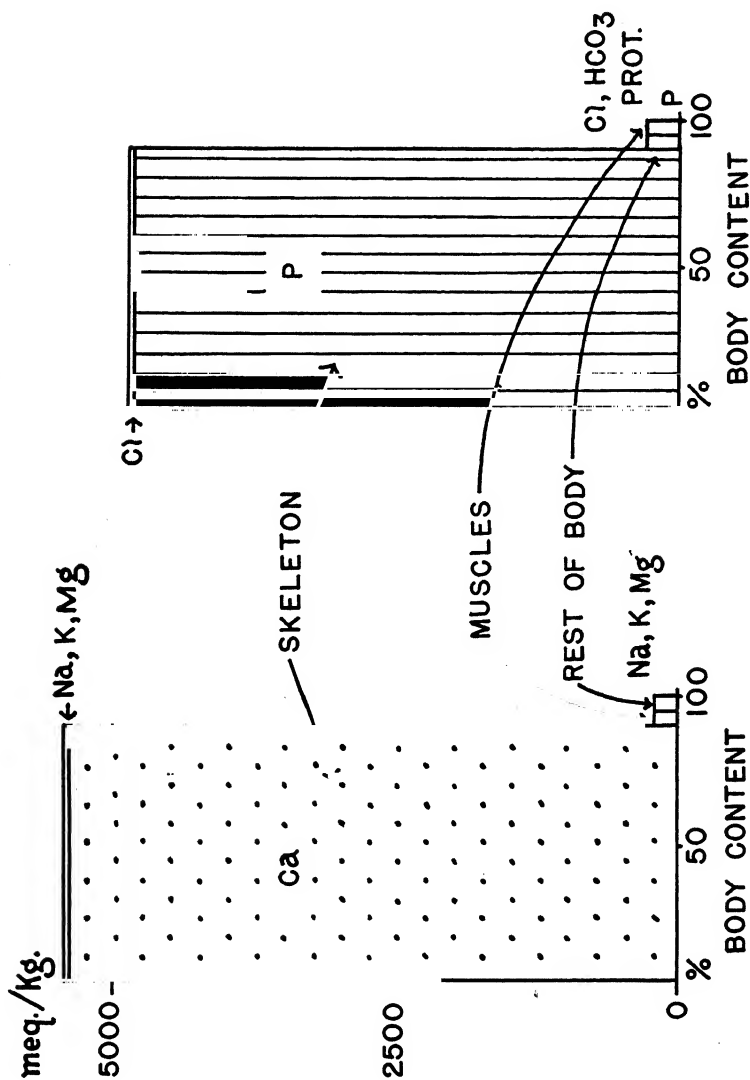
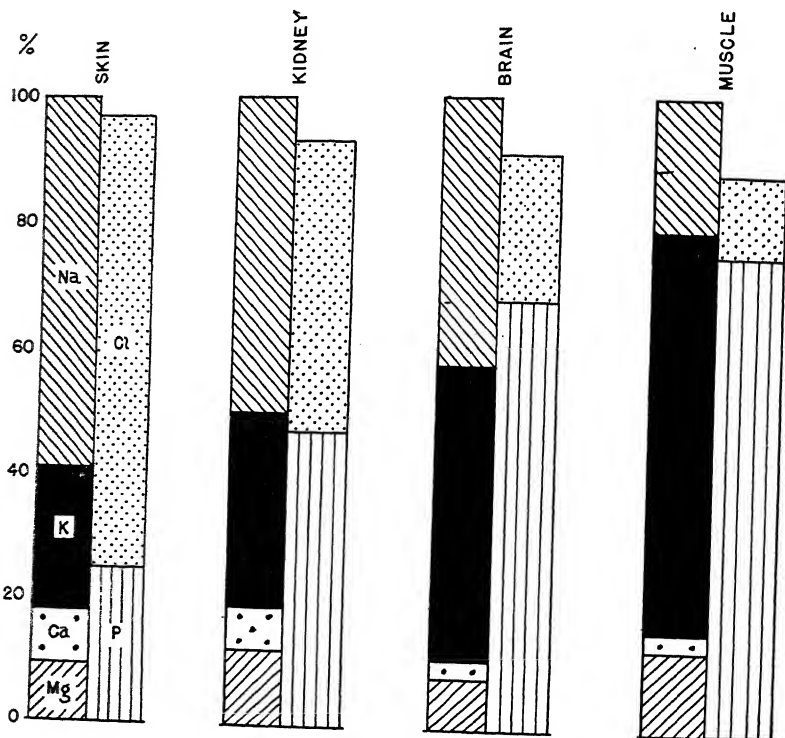


FIGURE 3. Distribution of Positive and Negative Equivalents in the Body (Fat-free).\*

\* From data in Tables 4 and 5. The ordinates represent the concentrations in the various organs, and the abscissae the per cent of the total positive or negative equivalents of the adult body. See text, page 21, for calculation of phosphorus.



The whole blood contains not more than 150 meq. of bicarbonate and the interstitial fluid (omitting plasma) not more than 300 meq. The amount within the cells is small. Thus only 1/15 of the carbonate + bicarbonate is found outside the bones, and hence is negligible in the calculation. The discussion of the electroneutrality of the blood cells (p. 24) showed that some of the positive elements must be in the form of proteinates, and therefore are not available to neutralize other anions.



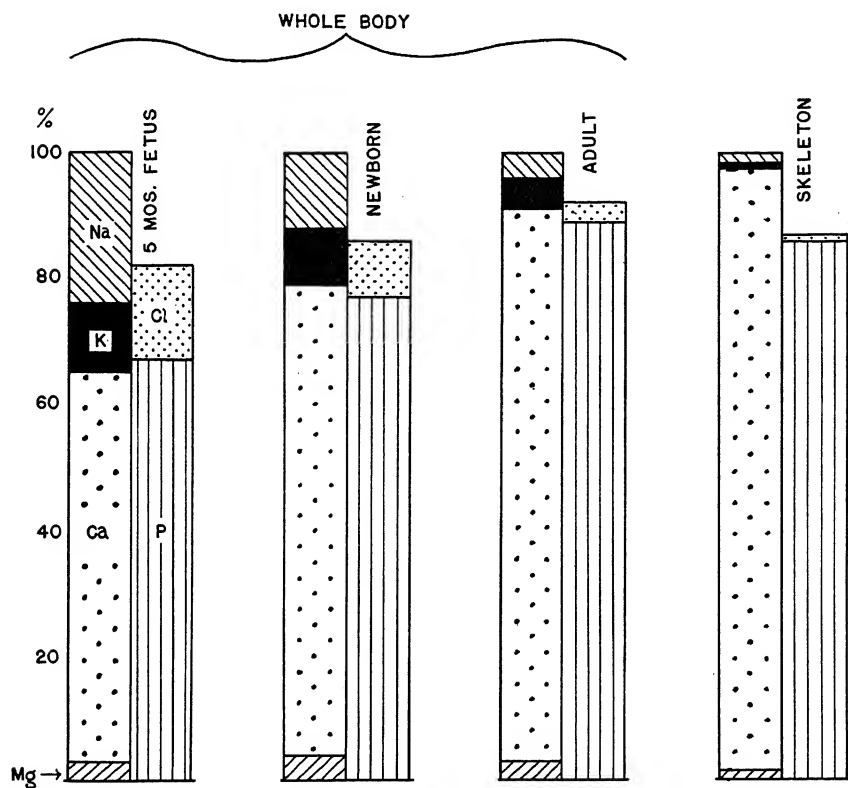
\* From data in Tables 3 and 4.

FIGURE 4. Percentage Distribution of

On the other hand the negative elements may also be united to protein. A discussion of proteins functioning as cations in the body as a whole is beyond our knowledge. Therefore, for the calculation of electro-neutrality the values given above serve only for orientation or approximation.

Figure 3 demonstrates the relative and absolute distribution in the body of the positive and negative minerals. The skeleton, because it includes practically all the calcium in the body, contains 91 per cent of

the total cationogens. The muscles account for 5.3 per cent, the blood cells and serum 1 per cent, and the remainder of the body, including all of the other organs, only 3 per cent of the positive eq. A similar preponderance of negative eq. also is found in the skeleton. Therefore the relationship between positive and negative minerals in the whole body



is essentially that of the skeleton. The bones contain 5,433 meq. of positive eq. per kg., and the whole body 1,136 meq. per kg. In contrast it has been shown that blood cells and muscles contain only about 180-190 meq. of cations per kg. of water. A rough graph of the organs for which adequate data are available indicates that a similar concentration obtains in the other organs also, and thus demonstrates osmotic equilibrium throughout the body, except the skeleton. Here the salts are in a solid phase, and hence cannot be calculated upon a water basis.

The values for the minerals in the bones are so great that when they

are graphically represented in comparison with the other organs as above, they obscure the details of the latter. To obtain an insight into relative structure, the mineral composition of the skeleton and other organs has been plotted separately on a percentage basis in Figure 4. This graph of the data in Tables 3 and 4 shows impressively the relationships of the positive and negative mineral elements in the organs and the whole body. The minerals were calculated in terms of equivalents. The anion value assigned to phosphorus was based on the total phosphorus and probable distribution of its fractions, and serves only for orientation (see p. 21). The data for the organs not given were regarded as not sufficiently reliable to warrant a graph.

A glance at Figures 3 and 4 is sufficient to establish the essential equality of the positive and negative minerals in the body and all the organs. The deficits in electronegative eq. are, of course, accounted for by proteinates, carbonates and undetermined anions. It is also obvious that the structure of electroneutrality varies from organ to organ. In the blood serum, sodium is the predominant cation, in the corpuscles potassium, and in the skeleton calcium is the main cationogen. Neutrality is attained in fluid, cell and solid bone, but by different mechanisms in each.

The four organs which have been taken as representative (omitting the skeleton) have been so placed in Figure 4 as to emphasize the following relationships: (1) when the phosphate is increased the chloride is diminished, showing inverse relationship between these anions; (2) when the potassium is increased the sodium is diminished; when the magnesium is increased (with the exception of brain) the calcium is diminished, showing that between similar cations there is also an inverse relationship. However, the  $[\text{Na}^+] + [\text{K}^+]$  and the  $[\text{Ca}^{++}] + [\text{Mg}^{++}]$  form relatively constant fractions of the cations. Stated in another way, Figure 4 demonstrates the inverse relation between  $[\text{Na}^+] + [\text{Cl}^-]$  and  $[\text{K}^+] + [\text{HPO}_4^-]$  and  $[\text{H}_2\text{PO}_4^-]$ . Further, it was previously shown that there is usually an inverse relationship between  $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$  in blood plasma. Although the status of the carbonates in organs is not well defined, the anions unaccounted for in Figure 4 are greatest when the  $[\text{Cl}^-]$  is smallest. Therefore it is not improbable that the carbonates show a similar relationship to chloride in the organs as well as in blood.

#### RELATIVE MINERAL COMPOSITION OF INFANT AND ADULT

The changes in the mineral composition of the body resulting from growth may be assessed from two viewpoints. The first involves the relative amounts of the various components, which measure the alterations in chemical structure. The amounts of the minerals per kg. of fat-free substance at different ages furnish the data for such considera-

tions (see Table 5). The second viewpoint involves the absolute amounts of minerals stored in the body (see Tables 3 and 4), which are a measure of the requirements for growth. The relative growth in minerals is discussed below, and the absolute growth is considered under *Mineral Accretion* (p. 47).

**Calcium and phosphorus.**—From analysis of Figure 4, it is clear that the calcium and phosphorus become relatively richer as the body develops. The increment in calcium and phosphorus has long been known to be associated with the rapid deposition of bone beginning in the last three months of fetal life. The weight ratio of Ca/P increases only from 1.6 in the five months fetus to 1.75 in the adult. These values are close to the ratio in bone, which is the same at all ages. But the other organs contain a much greater proportion of phosphorus. Therefore these ratios indicate that nearly all the phosphorus is combined with calcium in the bone. Further, in spite of the great growth of muscle, the phosphorus in the muscles constitutes a smaller fraction of the total phosphorus in adults than in the new-born.

**Magnesium.**—The increase of magnesium, both absolute and in relation to the rest of the body, is less rapid than that of calcium and phosphorus, but greater than that of any other element. It is found in the largest amount in the bones, but the muscles also contain a large share. Magnesium may occur in conjunction with phosphate, carbonate, or a complex salt of calcium phosphate. The constancy of the magnesium in the bone of many species<sup>80</sup> points to the possible constancy in human bone at various ages. Hammett<sup>39</sup> has reported such data for the rat. The following calculations are made in the absence of direct data, to test the deduction that magnesium is a constant fraction of bone salts of man.

Calcium in adult skeleton = 1150 gm.

Magnesium in adult skeleton = 11 gm.

Ratio of Mg/Ca = 0.01

Calcium in body of new-born = 23.6 gm.

Correcting for soft parts and assuming the same proportion of Mg/Ca as in adult,

Mg in skeleton of new-born =  $23 \times 0.01 = 0.23$  gm.

Magnesium in muscles of adult = 0.23 gm./kg.

Muscles of new-born = 0.77 kg.

Assuming the same proportion of Mg in muscle of new-born as in adult, Mg in muscles of new-born =  $0.77 \times 0.23 = 0.17$  gm.

Total Mg in new-born = 0.70 gm.

Mg in remainder of body (total - skeleton - muscles) =  $0.70 - 0.23 - 0.17 = 0.30$  gm.

Weight of new-born = 3.0 kg.

Weight of skeleton of new-born = 0.43 kg.

Weight of muscles of new-born = 0.77 kg.

Weight of remainder of new-born (total - skeleton - muscles) =  $3.0 - 0.43 - 0.77 = 1.8$  kg.

Weight of remainder of new-born on fat-free basis = 1.6 kg.

Mg per kg. of fat-free remainder of new-born =  $0.30 \div 1.6 = 0.18$  gm./kg.

This value approximates the average composition of the remaining tissues (except blood) of the adult (see Table 5). On the basis of the assumptions, it can be concluded that the distribution of the magnesium in the bones and tissues of the body is nearly the same in the new-born as in the adult.

Although the magnesium content of the bone salts is constant, 3.0 gm./kg., the fresh skeleton shows different proportions because of the differences in water content. On the basis of the same data the magnesium content of the skeleton of the new-born is 0.54 gm./kg. of fresh bone, and that of the adult is 0.95 gm./kg. of fresh bone, or 1.05 per cent of the fat-free bone. This explains the increasing proportion of magnesium in the whole body as it matures, from 0.18 to 0.36 gm./kg. of fat-free weight.

The ratio of Mg/Ca has been taken as an example of the way in which ratios and the data in Table 5 may be applied to specific problems. The relative amounts of P/S/N, or Na/K, or any others, may be similarly utilized to demonstrate their interdependence.

**Chlorine.**—The analyses with regard to chlorine are quite variable. The youngest fetus, according to Rosemann<sup>61</sup> (Tables 3 and 5) contains 0.25-0.27 per cent, and as the fetus grows the chlorine concentration diminishes. The average value for the new-born is 0.18 per cent, and for the adult 0.12 per cent. Magnus-Levy<sup>73</sup> estimated the total chlorine in the adult by adding to his partial analyses Wahlgren's values for the skeleton (and skin). His calculation for the fraction of the body chloride in the bones is 23 per cent of the total; and Klose<sup>61</sup> found 22 per cent in the normal infant. Calculated on the fat-free basis, the values for fetus, infant and adult come into closer agreement.

If one considers that the chlorine occurs, so far as is known, mainly in the body fluids, it seems logical to calculate the chloride on the fluid basis. Such calculations, given in Table 7, show that the fetus at all ages contains approximately 0.27 per cent chloride; the new-born 0.25 per cent;\* the adult 0.21 per cent: these values equal, respectively, 75, 70 and 59 meq./kg. of body water. These calculations are essentially in agreement with the newer data of Iob and Swanson<sup>54</sup> calculated on this basis.

It is well known that in fluids of high proteinate concentration the  $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$  are correspondingly reduced. The  $[\text{Cl}^-]$  of cerebrospinal fluid is 125 meq./kg. of water, but of the blood serum 113 meq./kg. of water; and in the blood cells the  $[\text{Cl}^-]$  is 80 meq./kg. of water. In whole blood the  $[\text{Cl}^-]$  is approximately 78.0 meq./l., or 100 meq./kg. of water. But the muscles with their high protein content contain still less  $\text{Cl}^-$ , 14 meq./kg., or 23 meq./kg. of water. If part of

\*Klose has reported lower values in the new-born on this same basis.

Table 7.—Chlorine Content of the Body.

Author *	Age	Body Weight (gms.)	Chlorine Content	
			Total Body (%)	Water Basis † meq./kg.
Rosemann	Fetus	111	0.25	77
Rosemann	Fetus	248	0.27	85
Michel	Fetus	445	0.24	77
Hugounenq	Fetus	522	0.24	77
Hugounenq	Fetus	570	0.27	88
Hugounenq	Fetus	800	0.20	68
Rosemann	Fetus	841	0.22	74
Langstein-Edelstein	Premature	960	0.28	97
Michel	Fetus	1024	0.29	102
Hugounenq	Fetus	1165	0.21	74
Hugounenq	Fetus	1285	0.22	80
Rosemann	Fetus	1339	0.22	80
Langstein-Edelstein	Premature	1420	0.23	85
Camerer-Söldner	New-born	2476	0.18	74
Camerer-Söldner	New-born	2616	0.18	74
de Lange	New-born	2680	0.19	77
Camerer-Söldner	New-born	2683	0.15	59
Hugounenq	New-born	2720	0.15	59
Camerer-Söldner	New-born	2755	0.18	74
Camerer-Söldner	New-born	3048	0.19	77
Hugounenq	New-born	3300	0.15	60
Michel	New-born	3335	0.19	77
Camerer-Söldner	New-born	3348	0.18	74
Magnus-Levy ‡	Adult	70 kg.	0.122	59

\* Cited by Czerny and Keller.<sup>18</sup>

† These values were calculated from Fehling's data on the water content of the fetus at different ages.

‡ Magnus-Levy.<sup>73</sup>

this water, approximately  $\frac{1}{4}$  (see under *Muscles*, p. 30) is intercellular, and of high  $[\text{Cl}^-]$ , the intracellular water must be very low in  $\text{Cl}^-$ . Because no accurate analysis of this portion is available, the chloride will be calculated for the muscles *in toto*.

In the whole body of a 70-kg. adult there are 41 kg. of water. If the average  $[\text{Cl}^-]$  is 58 meq./kg., the total = 2380 meq. The muscles contain 21 kg. of water. At 23 meq./kg. of water, the muscle chloride =  $21 \times 23$ , or 480 meq. But the total minus that in the muscles = 1900 meq., or for the remaining 20 kg. of water, 95 meq./kg. of water. Thus the remainder, the non-muscle body water, has approximately the same  $[\text{Cl}^-]$  as whole blood. This seems reasonable for the subcutaneous tissue, skin and skeleton contain a fluid relatively richer in  $\text{Cl}^-$  than do muscles.

Corresponding calculations for fetus and new-born yield essentially similar results because of the relatively smaller amount of muscle and protein, and the larger amount of extracellular, high  $[\text{Cl}^-]$  body water.

Thus the variable chloride content of the body at different ages, which has previously been an enigma, is explained on the basis of its constant concentration in the body fluids, and their decrease per unit of body weight with maturity.

**Sodium.**—One may attempt to find the change in distribution of the monovalent cations in the body at various ages by procedures similar to those used for chlorine. It is known that these cations are distributed principally in the body fluids. Further, they occur in part as proteinates. The sodium occurs principally in the extracellular body fluids (as in serum) and the potassium is located within the cells (as in muscle or red blood cells).

The  $[\text{Na}^+]$  of the body, calculated on a water basis, equals, in the youngest fetus, 125 meq./kg. of water, in the new-born 97, and in the adult, 67. Let us compare these values with blood. The serum contains 154 meq., the cells 15 meq./kg. of water; the whole blood 88 meq./kg. or 125 meq./kg. of blood water. To understand why the  $[\text{Na}^+]$  of the body water should be so much lower than that of serum, the distribution in the body must be investigated. The two main depots for sodium, each of which contains about one-third of the total, are the skeleton and the muscles, in both of which it occurs largely in interstitial fluid. If the muscle, which is poor in sodium, but of large bulk, is considered separately, the  $[\text{Na}^+]$  of non-muscle water can be calculated as for  $[\text{Cl}^-]$ . Hill and Kupalov<sup>50</sup> have given the muscle  $[\text{Na}^+]$  as 44 meq./kg. of water. By simple computation the non-muscle body water contains 88 meq./kg. for the adult, and 116 for the infant. (In the infant considerable sodium is located in the cartilage.) So the sodium in the rest of the body, like that in the muscles, is found in the extracellular fluid, and varies with the proportion of extracellular to intracellular fluid.

Sodium and chlorine are the only two elements which are proportionately less in the adult than in the new-born. This is because both are prominent in the structure of intercellular fluid, which diminishes with maturity.

**Potassium.**—Calculated as for sodium in terms of body water, the  $[\text{K}^+]$  of the body equals 52 meq./kg. of water in the youngest fetus, 63 at birth, and 95 in the adult. These values may be compared to that of blood. The serum  $[\text{K}^+]$  is only 5 meq./kg. of water, but that of the cells is as much as 170, and the whole blood 47 meq./l. or 65 meq./kg. of blood water. The red cells, although rich in potassium, contain only 6 per cent of the total potassium in the body. The muscles contain 72 per cent, and no other organ contains as much as the blood. The  $[\text{K}^+]$ /kg. of muscle water equals 132 meq.<sup>50</sup> Here the case is just the reverse of that of sodium; the  $[\text{K}^+]$  of muscles is greater than in the average of other organs. When the muscle potassium is subtracted from the total, the  $[\text{K}^+]$  in the remaining body water is found to be 54 meq./kg. in the adult, and 39 in the new-born. Hence this value, like that of sodium, resembles whole blood more nearly than cells or serum.

The potassium forms a nearly constant fraction of the fetus, new-

born and adult, for its rate of increase and that of sulfur are, of all the minerals, most nearly parallel to increase in body weight. The two are closely associated with protein rather than body water.

**Sodium and potassium.**—The potassium of the fetus is about the same in weight as the sodium, or one-half the amount in equivalents. As the body develops, the potassium increases more rapidly than the sodium, and in the adult is more than twice the weight of the latter. Moreover, per unit of body weight, the  $[\text{Na}^+]$  is diminished while the  $[\text{K}^+]$  is increased. The total body water is richer in potassium and poorer in sodium because of the greater proportion of muscle in the adult. To bring out the significance of these alterations in the body water it is necessary to consider this fluid, in its two main fractions, namely the intracellular and extracellular water, in accord with the thesis developed by Gamble, Ross and Tisdall.<sup>29</sup>

So far the muscles have been considered as a whole. If the assumption is made that the sodium is extracellular, or intercellular, and the potassium is intracellular, the muscle water can be divided into these two fractions. (The small amount of potassium outside and of sodium inside the cells may be assumed to balance each other.) The  $[\text{K}^+]$  is 132 meq. and the  $[\text{Na}^+]$  is 44 meq./kg. of muscle water.<sup>29, 50, 88f</sup> The sodium represents 25 per cent of the sum of the two, and if the cells are in osmotic equilibrium with the surrounding fluid this percentage must represent the amount of water outside the muscle cells.

In the following table the  $[\text{Na}^+]$  and  $[\text{K}^+]$  in the whole body are given in meq./kg. of water. The percentages of extracellular and intracellular water are calculated on the assumption that the potassium is wholly within the cells and the sodium without.

	$[\text{Na}^+]$ (meq./kg. of $\text{H}_2\text{O}$ )	$[\text{K}^+]$ (meq./kg. of $\text{H}_2\text{O}$ )	$[\text{Na}^+] + [\text{K}^+]$ (meq./kg. of $\text{H}_2\text{O}$ )	Extra- cellular fluid (%)	Intra- cellular fluid (%)	Muscle weight (% of body)
Fetus .....	125	52	177	71	29	
New-born .....	97	63	170	63	37	25
Adult .....	67	95	162	42	58	43
Adult (corrected for intracellular sodium)				30	70	

The sum of the two cations in the total body fluid equals 162 meq./kg. of water. This value is not only nearly the same for the whole body throughout life, but for the muscles also. Thus the osmotic pressure remains constant. The calculation shows further that as life progresses and the cells increase, an ever increasing amount of the body water becomes intracellular: in the fetus only 29 per cent, and in the adult 58 per cent. This value seems too low, although the proportion of intracellular water in muscle (75 per cent) probably exceeds that in the body as a whole. Inasmuch as more sodium is intracellular than potas-



sium intercellular, the best tentative allocation of the body water seems to be 70 per cent intracellular and 30 per cent extracellular. This calculation is also in agreement with the known weights of muscle at these various ages. The experimental evidence has been extended by analysis of the whole bodies of rabbits, dogs and a monkey.<sup>41</sup>

Crandall and Anderson<sup>16</sup> used NaSCN to estimate the amount of interstitial fluid. This is rapidly distributed throughout the body in the fluids but not in the cells, except red blood cells. It is slightly more concentrated in serum than in other fluids. Stewart\* has found that this concentration in serum varies from individual to individual but is constant for a given case. The amount of intercellular fluid thus estimated averages 18.8 per cent of the body weight, or 27 per cent of the body water.

Thus by consideration of the sodium, potassium and chlorine in terms of body water, it becomes apparent that the previously unexplained values conform throughout life to the maintenance of both osmotic and ionic equilibrium.

**Excess of positive mineral equivalents.**—The relation between the positive and negative ionogens in the composition of the adult body has been discussed on page 36. Limiting this discussion to the mineral equivalents only, the values for fetus, new-born and adult, expressed in meq./kg. of fat-free body, are as follows:

	Negative minerals (meq.)	Positive minerals (meq.)	Excess positive minerals (meq.)
Fetus .....	380	475	95
New-born .....	526	610	84
Adult .....	1052	1146	94

These values are represented graphically, on a percentage basis, in Figure 4. In this calculation the phosphorus has been given a combining value of 2.7 eq./mol, and the sulfur omitted. It is plain that, per unit of weight, there is a small and constant excess of positive minerals, although both anionogens and cationogens increase with age. This increasing concentration of minerals is due to the increasing preponderance of calcium and phosphate in solid phase in bone. This fact prevents calculation on a basis of body water. However, as these exist in approximately equivalent amounts, they cancel out of the sum. Hence electroneutrality of the body fluids depends mainly upon the sodium, potassium and chloride, and in addition, on that fraction of the phosphorus not found in the bones. The mineral anion deficit is made up by carbonates, proteinates and unknown acids. Thus, from the point of view of mineral content, the relationship between positive and negative elements is essentially constant in the body at all ages.

\* Dr. J. D. Stewart, personal communication.

## MINERAL ACCRETION

It would be highly desirable to trace the absolute increases of the various inorganic constituents in the body and organs. In this way it would be possible to determine the magnitude of the daily or yearly retentions necessary to maintain normal body structure. Adequate chemical analyses of the body and organs at various ages would permit construction of a graph of increase in each of the elements throughout

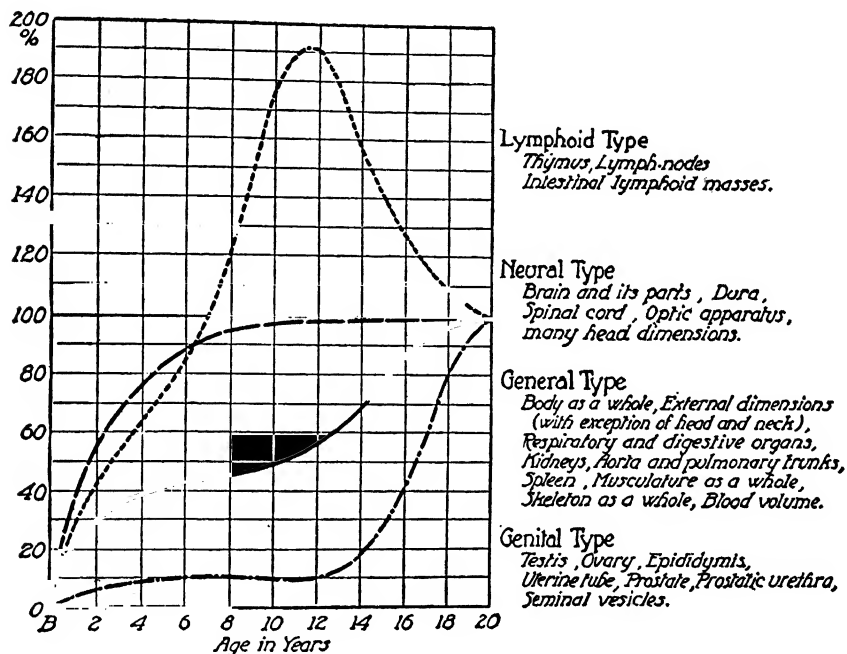


FIGURE 5. Rate of Growth of Different Organs.\*

\* Figure 73 of Scammon,<sup>40</sup> reproduced by permission of the University of Minnesota Press. "A graph showing the major types of postnatal growth of the various parts and organs of the body. The several curves are drawn to a common scale by computing their values at successive ages in terms of their total postnatal increments (to twenty years)."

life. Unfortunately only the gross body weights and heights at all ages are accurately known. The weights of the organs in infancy and maturity are presented in Table 4. The relations of the weights of the organs to the whole body and to one another at the two ages manifest different rates of growth. In order to compute the growth of the various body organs at different ages the graph given by Scammon<sup>40</sup> has been utilized (see Figure 5). The muscles and skeleton increase more rapidly than the whole body, the skin at about the same rate.

These three however, comprise 85 per cent of the total weight, and therefore deductions based upon them would represent practically the whole body. Even if the weights of the organs and also their chemical composition at different ages were known it could not affect the total materially.

Students of growth have been interested largely in the ash and water content, certainly since Liebig's time, and Lawes and Gilbert, Voit, Bunge, Abderhalden and many others have made numerous contributions to the knowledge of ash content of the higher mammals and laboratory animals. Extensive accounts of these have been given by Aron,<sup>3</sup> Forbes and Keith<sup>20</sup> and Armsby and Moulton.<sup>2</sup> Moulton<sup>81</sup> has based his hypothesis of "chemical maturity" largely upon such data. He finds that in all mammals, on a fat-free basis, the ash increases and the water decreases rapidly during gestation and after birth until 4.4 per cent of the total age is reached. After that the ratio of ash and water to body weight, on a fat-free basis, remains nearly constant. By such a computation "chemical maturity" is reached in the human species at three and a half years.

Inasmuch as 83 per cent of the ash lies in the skeleton, the ash of the whole body is dependent largely upon that organ. Therefore Moulton's law should be altered to read: "Mammals reach chemical maturity of the skeleton at different ages, but these ages are a fairly constant part of the total life cycle. Thereafter the ash of the skeleton forms a constant proportion of the body ash."

That such is not the case for the integument is proved by the studies of Brown.<sup>8</sup> He found that the concentrations of ash, calcium and magnesium in the skin are greatest at birth, that they decline rapidly until about puberty when a slow, continuous, secondary rise sets in, and that in old age the amounts approach those found at birth.

The growth of the muscles may be calculated from Scammon's data. The greatest increase in muscle tissue appears to occur after the skeleton is "mature," and reaches its greatest bulk after puberty.

In addition to the chemical composition of the organs of the adult summarized in Tables 4 and 5, there are available the analyses of the skeleton, skin, subcutaneous tissue, muscles, and a fourth group, "internal organs," of normal and edematous new-born infants, given by Klose,<sup>61</sup> and of a few sick infants.<sup>104, 105</sup>

Two methods are available for estimating mineral increases. The first comprises the measurements of the actual retentions. This method is discussed in Chapter 14, under *Balance Studies*. The second method, that of computing the rate of increase in minerals from the body composition, was proposed by Herter,<sup>45</sup> who stated:

"I have calculated that the average yearly accretion of calcium oxide by the skeleton between the third and sixteenth year is 51.6 grams.

"The above result was reached by the following data:

"Estimate of the average yearly addition of calcium (as calcium oxide) to the human skeleton between the third and sixteenth year.

Weight of body at 3 years .....	16 kilos
Weight of body at 16 years .....	50 kilos
Estimated weight of skeleton in per cent of total at three years.....	15
Estimated weight of skeleton in per cent of total at 16 years.....	17
Weight of skeleton at 3 years .....	2.4 kilos
Weight of skeleton at 16 years .....	8.5 kilos

Undried bone contains 22 per cent of bone earth.

Skeleton of 2.4 kilos contains 0.53 kilo of bone earth.

Skeleton of 8.5 kilos contains 1.87 kilos of bone earth.

Accretion of bone earth by skeleton between the ages of 3 and 16 years equals 1.34 kilos.

Bone earth contains approximately 84 per cent calcium phosphate.

Bone earth contains approximately 13 per cent of calcium carbonate.

1.34 kilos bone earth contain 1.125 kilos calcium phosphate.

1.34 kilos bone earth contain 0.175 kilos calcium carbonate.

1.125 kilos calcium phosphate contain 0.60 kilos calcium oxide.

0.175 kilo calcium carbonate contains .007 kilos calcium oxide.

Total calcium oxide in 1.34 kilos of bone earth = 0.67 kilos calcium oxide.

"This represents the accretion of calcium oxide during thirteen years of skeletal growth. The yearly average accretion of calcium oxide in skeletal growth between these years therefore equals 0.0516 kilos or 51.6 grams."

The results of calculations of accretions, by Herter's method, from birth to 24 years, indicate an average daily retention in milligrams and milliequivalents of the elements as follows:

	Ca	Mg	Na	K	P	S	Cl
Mg. ....	130.	13.	6.7	14.	75.	13.	9.1
Meq. ....	6.5	1.1	0.29	0.36	6.5	?	0.25

Excess positive minerals = 1.4 meq. per day.

The data computed year by year for the calcium, based on the variable increase in weight, increase in per cent of skeleton and increase in per cent of calcium and the "chemical maturity," are given in Table 8 and Figure 6. From inspection of the values computed for calcium it is clear that during the first year the increase of calcium is very great, and that the increments per kg. diminish until maturity. However, owing to the larger size, the total retention increases from the fourth year until puberty.

The values we have calculated are greater than those which were computed by Herter. Leitch<sup>69</sup> has made a calculation of the skeletal content of calcium. She made the assumption that "the fresh weight of the skeleton was a constant proportion of body weight and the calcium content varies inversely as the water content of the skeleton." She does not provide the data used for calculation of the water content. The calcium content of adolescent boys is calculated as 36 gm./kg. of

body weight (presumably greater for the adult). This amounts to over 2200 gm. of calcium, or about twice the values we compute. This large amount of calcium would represent an ash of over 5 kg. for a normal adult, again twice the value we have given. Our values calculated as per cent of the body weight compare with those of Sherman,<sup>97</sup> but again are less than one-half of Leitch's values. Although her values were chosen to approximate the maximum, and we may have erred in being too conservative, the two sets of computations frankly cannot be brought into agreement.

Table 8.—Calcium Content of the Body at Different Ages.\*

Age (yrs.)	Weight (kg.)	Growth (kg./yr.)	Skeleton (% of total weight)	Calcium		Calcium Accretion		
				(% of skeleton)	(gm.)	(gm./yr.)	(mg./day)	(mg./kg. /day)
Birth	3	7	13.5	5.1	20			
1	10	7	14.5	5.8	82	59	163	25
2	13	3	15.5	6.4	122	40	110	10
3	14.5	1.5	16.0	6.8	155	33	90	6.6
4	15.5	1	16.5	7.3	180	25	68	4.7
5	17	1.5	17.0	7.7	218	38	105	6.5
6	10	2	17.5	8.1	268	50	138	7.7
7	21.5	2.5	17.5	8.5	320	52	142	7.1
8	24.3	2.8	17.0	8.8	370	50	138	6.1
9	27.3	3	17.8	9.0	428	58	158	6.9
10	30	3	17.5	9.2	482	54	148	5.2
15	50	4	17.5	9.4	830	69	190	4.7
22	64	2	17.5	9.5	1000	24	65	1.1
Adult	70		17.5	9.5	1160			

\* Calculated from the data in Table 3 and Figure 5.

The data in the first four columns have been calculated from a plot of three variables against age, weight, per cent of total weight representing skeleton (fresh weight), and per cent of calcium in the skeleton. The figures in the fifth column represent those in the first, times the third, times the fourth. In calculating the calcium growth per year the average weight of the periods before and after was used.

The increase in minerals depends partially upon the intake; greater retentions follow greater intakes. Growth may be limited by the calcium and/or phosphorus intakes.<sup>98, 99, 100, 122</sup> Hence the values we have given represent only average individuals; they do not define optimal or maximal growth.

Similar calculations can be made for phosphorus, because of the constant relation of this element to calcium in bone. If the calcium values are divided by 2.15 the phosphorus values in the bones are obtained, and represent 80 per cent of the phosphorus in the body. If greater accuracy is desired, the muscle phosphorus can be similarly calculated, and half the remaining 20 per cent accounted for. A plot of the variable body weight, per cent of muscle (25-43 per cent) and per

cent of phosphorus in muscle (1.5-2.2 mg. per 100 gm. of fresh muscle) shows an additional retention by the muscles of 5 mg. of phosphorus daily in early childhood, and 10 mg. in adolescence. It is not felt that the data are sufficiently well established to justify further elaboration of such estimates. Similar values can be computed for the other elements, but these also have been omitted, for the same reason.

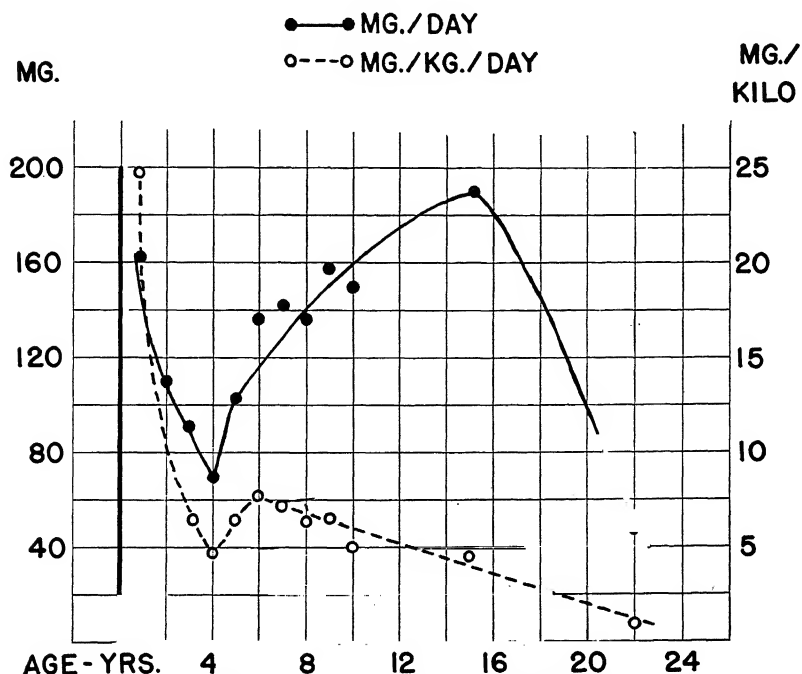


FIGURE 6. Rate of Calcium Growth.\*

\* From data in Table 8.

Estimates of the "composition of growth" have been made by comparison of mineral retentions to the composition of the body at birth, or to increases in nitrogen, to phosphorus, sulfur, or body weight. Such correlations cannot be expected to be valid, for it is found above that the body composition is altered for each stage of development, and that the retentions likewise vary with each period investigated.<sup>18p, 38, 107, 121</sup> The literature is full of fruitless efforts to correlate mineral growth with protein or weight increases. It is only when the distribution in organs is known that mineral deposits can be rationally allocated.

**Excess positive mineral equivalents in growth.**—The retention of more positive than negative mineral equivalents occurs throughout the

growth period. Calculations of this excess can be made on the daily or yearly basis. It has been shown above that each fat-free kg. of body contains about 84-94 meq. excess of positive minerals; therefore this increase must be directly proportional to the weight increase. A baby grows 7 kg. the first year (or 6, fat-free) and hence adds 570 meq./yr. of excess positive minerals, or 1.6 meq./day.

A previous attempt was made to evaluate this excess during the growth of a baby from birth to one year of age.<sup>101</sup> The analysis of ash of the fetus was the basis for the calculation. The assumption was made that the infant increased 6.6 kg. in body weight, from 3.3 to 4.0 in per cent of ash, representing a 300 gm. increase in ash, and that the ash was of constant composition. The cationogen excess stored was calculated to be 5.8 meq. per day, with the use of 1.8 eq. per mol as the combining value of phosphorus, and of 2 for sulfur. If we use 2.7 for phosphorus and omit sulfur, the excess calculates to 2.4 meq. per day, which is in fair agreement with that above. However, in the light of further experience, the assumption that the ash is of constant composition makes such a calculation less desirable than the present method.<sup>101</sup>

A third method of determination, the measurement of intake and output in balance studies, yields similar but slightly larger values. (See Chapter 13. p. 300.)

The bicarbonate, carbonate, proteinate, undetermined acids and phosphate not found in bone neutralize that fraction here called excess positive minerals. These calculations show only the order of magnitude of the excess of the electropositive minerals which must be retained daily during the period of growth to insure the normal structure of the body.

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## Chapter 3

### Secretions and Excretions

#### GENERAL CONSIDERATIONS

The mineral structure of the body and organs was detailed in Chapter 2; that of the secretions will now be considered. The secretions are discussed separately, for they are not only large in amount, but are of specific and divergent composition and function. A later section of the chapter will show their collective effect on the body fluid as a whole.

#### Classification of Secretions and Excretions

We call secretions those glandular products which serve a further useful purpose in body metabolism. We reserve the term excretions for those fluids which remove waste products from the body. This customary differentiation is more or less arbitrary though often useful. Both excretory and secretory functions represent selective absorption and selective output, and are often intermingled in the same organ. Therefore, in a true sense, the excretions may be said to be secretions whose primary function is to regulate the homeostasis of the body.

**Secretions.**—For convenience we have divided the secretions into three classes.

1. *Alimentary secretions* include saliva, gastric juice, bile, pancreatic juice and intestinal juices. These are all poured into the gastro-intestinal tract, and, after their special functions are performed, are either partially or wholly reabsorbed.

2. *Internal secretions* include products of the ductless glands: the adrenals, hypophysis, pancreas, thyroid, parathyroid, thymus, pineal and sex glands. These are of interest to us not because of the minerals they contain, but because of their effect on mineral metabolism. They are discussed in the next chapter.

3. *External secretions.* Milk, because of its unique chemical composition, its function and its transitory nature, forms a special problem. The external sebaceous and mucous secretions have slight relation to mineral metabolism.

**Excretions.**—The excretions also have been divided into three classes.

1. *Urine and feces.* The urine especially, and the feces secondarily, by their excretion of water and salts, serve to regulate the various mineral equilibria of the body.

2. *Sweat.* The function of sweat is primarily to regulate heat equilibrium through the evaporation of its contained water. The mineral content is its incidental concomitant, although it may reach important proportions.

3. *Carbon dioxide.* The lungs excrete the major portion of the  $\text{CO}_2$  formed in metabolism and a smaller part of the water. The excretion of  $\text{CO}_2$  depends upon the amount of carbon burned in the body, and therefore is primarily related to energy metabolism. Insofar as the transportation of  $\text{CO}_2$  by the blood is important in relation to the acid-base and osmotic equilibria, it has been considered briefly in Chapter 13, under *Mechanism of Acid-Base Equilibrium*. Further relations have been summarized under *Bicarbonate* (p. 130).

### Secretory Mechanism

The problem of how cells may manufacture materials and absorb and excrete them is fundamentally one of general physiology. Whether secretion is accomplished by a transudation of the minerals from the blood or by the secretory activity of the glands need not be discussed in detail here. Peters has devoted a large part of his book, "Body Water,"<sup>82</sup> to a discussion of this problem.

The extracellular fluid is protein-free and the blood serum contains protein. Protein affects the activity of water, *i.e.*, causes increase in the osmotic pressure of the fluid containing it. This pressure, called oncotic, is balanced by the hydrostatic pressure of the blood stream, and these opposing forces determine the transudation of materials through the blood vessels and their selective reabsorption.

Selective secretion and selective absorption represent the same phenomenon looked at from two different sides. This means that the membranes involved are permeable to certain ions or substances in one direction only. Thus gastric mucosa allows  $\text{H}^+$  and  $\text{Cl}^-$  to pass into the stomach but not in the reverse direction, and the intestinal tract allows no secretion of dextrose into the lumen, but absorbs it from the intestinal contents into the blood. Such one-way permeation or secretion has been cited by the vitalists as an illustration of the thesis that certain activities of cells are due to a special property inherent in living cells, a "vital force." Such cells operate under physical conditions of which we have at present no adequate description. That they must be, however, subject to the laws of physics and chemistry is evidenced by the following example in which this selective action is reversed. If an animal is

rendered hyperglycemic and in addition given hypertonic saline intravenously, glucose is secreted into the intestine.

In regard to kidney secretion, Ludwig took the position that the urine is formed in proportion to the effective arterial pressure in the kidney, and Heidenhain felt that further selective secretion or reabsorption by the cells of the tubules is necessary to explain the excretory mechanism. The evidence on both sides has been augmented through generations of physiologists until it is truly monumental, and the interpretation of the phenomena seems to be partly a matter of temperament. The same arguments have been pursued with regard to the other glandular secretions.

### Nature of Secretions

Secretions are fluids containing electrolytes and those organic compounds which are elaborated by the secreting cells. The specific mineral composition of the various secretions is given in Figure 7, and will be detailed later in the chapter. Although under stress the mineral contents of the secretions are not unalterable, they are of practically fixed and definite composition. The composition of excretions must, of course, vary widely, for the amount and character of the material excreted depends upon that ingested, and upon that which must be removed to maintain the constancy of the other body fluids. To illustrate: ingested foreign substances such as drugs, potassium iodide for example, may be found in saliva and milk, but by far the largest part is found in the urine. Perhaps the best example is water; that not needed is excreted in the urine whether the intake is one liter or six liters.

**Osmotic equilibrium.**—The relation of blood to secretions is of first importance, for blood supplies the minerals for their formation. All the alimentary secretions, except the hypotonic saliva, have approximately the same osmotic concentration as that of the blood, and are presumably in osmotic equilibrium with it. Only the loops of Henle in the kidney seem to be able to perform osmotic work, *i.e.*, concentration, and produce a fluid of higher osmotic pressure than the blood. In conditions which involve changes in the osmotic pressure of the blood, the composition of the secretions formed is correspondingly altered.

**Ionic equilibrium.**—The secretions derive their minerals from the blood, and their content may either closely resemble the configuration in blood or show marked deviation from it in either relative or absolute amounts of the various minerals. The secretions may contain minerals in greater concentration than the blood, as chloride in gastric juice and sodium and calcium in bile; or in lesser concentration, as sodium in gastric juice and chloride in bile; and also each differs from the others in the proportions of Na/K, Ca/Mg, etc. Thus is shown the selective permeability or secretory activity of the different glands in which these

juices are formed. The pattern of secretion is fixed in each gland (except the kidney) provided the blood remains constant. Alteration of any specific ion in the blood is reflected in the secretions, but to a different degree in each. The degree of alteration depends upon the capacity of the gland to secrete that particular ion, and is proportional to the original composition of the secretion. In other words, increase of an ion in the blood will be reflected only slightly in a secretion normally low in that ion, but to a greater extent in one in which it is normally high. Such changes may result from pathological conditions or injections of different salts, and are exemplified later. (See page 63.)

**Acid-base equilibrium.**—The acidities of the secretions, unlike their osmotic pressures, show wide divergences from that of the blood. All the other body fluids have a pH close to that of the plasma, but the secretions differ not only from this, but also from one another. This fact is illustrated in the following table, which is compiled from the literature. These differences in the digestive secretions depend primarily upon the proportion of cations to  $\text{Cl}^-$ , or more exactly, upon the relation of the sum of the cations to the sum of the anions. Except in the cases of gastric juice and bile, low  $[\text{Cl}^-]$  is accompanied by high  $[\text{HCO}_3^-]$ , and greater alkalinity results. These pH values are not invariant, but the fluctuations produced by ingestion of acid or alkali have not been shown to be significant. The acidity of the urine is a special case, and will be discussed later. The various enzymes in the secretions operate to maximal advantage at the pH of the juice in which they originate.

Secretion	pH	Secretion	pH
Pure fundic juice .....	0.92	Saliva .....	6.6-7.0
Mixed gastric juice .....	1.2-1.3	Milk .....	6.8-7.0
Urine .....	4.5-8.0	Hepatic bile .....	7.1-8.6
Sweat .....	4.6-6.0	Colon juice .....	7.6-8.0
Feces .....	5.6-7.8	Pancreatic juice .....	8.0
Bile .....	6.3-7.0	Pleum juice .....	8.6 (?)
Jejunum juice .....	6.5-7.1		

### Factors Affecting Volume of Secretions

When food and water are ingested, secretion of digestive juices takes place, and the amount of secretion is dependent upon the character and amount of materials ingested. But the volumes of the various secretions are affected by many other factors. A dictum coming from Liebig's time, called "The Law of the Minimum," applies to many phases of body activity, including secretions. It may be expressed in this case as follows: when there is a limiting factor the secretion is decreased in amount rather than altered in composition. This is well exemplified by the secretion of milk, but less well authenticated for the other secretions.

*Nervous and hormonal control.*—Secretion is affected by both nervous impulses and hormones. The studies of Pavlov showed that the mere sight and smell of food lead to a psychic secretion of the alimentary juices. It has long been known that stimulation of nerves can either increase or decrease the flow of saliva. Part of this influence may be attributed to vasomotor changes and part for want of a better description is said to be due to the action of secretory nerves. Further evidence of nervous control is shown by puncture of the floor of the fourth ventricle of the brain, called "salt puncture." This operation leads to an increased excretion of  $\text{Na}^+$  and  $\text{Cl}^-$  in the urine.

The rate of secretion of urine depends not only upon blood composition but also upon the volume and the pressure of the blood flowing through the kidney. Part of the nervous and hormonal control of secretion of urine may be related to the latter factors. Section of the nerves of the kidney leads to excretion of a large amount of dilute urine. After removal from the body and perfusion of the kidney, either alone or through a heart-lung preparation, urine is still secreted. This urine is extremely dilute. The antidiuretic effect of pituitrin is still demonstrable in such a kidney.

The effects of the internal secretions except for pituitary hormone depend largely upon their capacity to modify the minerals in the blood. They are discussed in the next chapter.

*Drugs and poisons.*—The actions of drugs and poisons have been studied extensively in regard to their effects on various secretions. These pharmacological and therapeutic problems lie beyond the scope of this book. Many examples come readily to mind. To mention a few: atropine, for instance, reduces the amount of sweat and morphine increases sweat but decreases all the other secretions; caffeine acts as a diuretic as do various salts of mercury; bitters increase gastric secretion.

Numerous drugs have been used to aid in studies of physiological functions. Examples are the use of cyanides which are supposed to have the specific effect of paralyzing the functions of the tubules in the kidney; and of sodium fluoride to poison the intestinal mucosa, in studies on absorption.

## ALIMENTARY SECRETIONS

### Saliva

The function of saliva is twofold; first, to start digestion by means of solution and action of ferments, and second, to lubricate the food for its passage through the esophagus. Saliva is secreted by the sub-maxillary, sublingual and parotid glands, and perhaps by the mucous membrane of the mouth. The secretions of the glands differ; that of the parotid is more watery, the others contain more mucin. The saliva



of one individual may show considerable variation from time to time, both in volume and composition.<sup>103</sup>

The nervous control of the glands is through cerebral and sympathetic nerves, which respectively dilate and contract the arterioles. Thus an accessible arena was offered to physiologists, especially since Heidenhain, to test their theories of specific secretion and filtration. Mathews states that stimulation of the cerebral nerves causes greater flow and more watery secretion than that produced by stimulation of sympathetic nerves. This difference may be attributed in part to the blood flow.

The flow of saliva may be started by psychic stimulation. This was shown by the classic experiments of Pavlov; the sight and smell of food causes a free flow of the secretion. It may also be inhibited psychically, as exemplified by the "ordeal by rice." If the suspect had guilty knowledge he was supposed to be unable to swallow dry rice.

The volume of saliva produced depends also upon the state of hydration of the body, and hence upon the amount of water consumed and the amounts excreted in urine and sweat. The food factors which influence the volume are quantity, dryness and length of chewing. The normal amount of saliva produced is 1500 cc. or more per day.

Local irritants increase the secretion of saliva. Among the drugs which increase the flow must be mentioned specifically pilocarpine and mercury. Salivation is one of the classic signs of mercury poisoning. Atropine causes diminution of secretion.

Saliva has a specific gravity of 1.002-1.008. The freezing point depression is  $\Delta = 0.28-0.40$  °C. Hence its osmotic pressure is far below that of the blood. The approximate mineral composition of the saliva is shown in the following table:\*

Average Composition of Mixed Saliva.<sup>84</sup>  
(Ash 0.219 per cent)

Cations	%	meq./l.	Anions	%	meq./l.
Na <sup>+</sup>	.02	9	Cl <sup>-</sup>	.04	10
K <sup>+</sup>	.10	25.8	P (H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> and HPO <sub>4</sub> <sup>--</sup> )	.018	10
Ca <sup>++</sup>	.006	3	HCO <sub>3</sub> <sup>-</sup>		10-15
Mg <sup>++</sup>	.002	2	CNS <sup>-</sup>	.01	
		40			38

It will be noted from the table that the solution is greatly hypotonic, and relative to the blood is low in [Na<sup>+</sup>] and [Cl<sup>-</sup>] and high in [K<sup>+</sup>] and [H<sub>2</sub>PO<sub>4</sub><sup>-</sup>] and [HPO<sub>4</sub><sup>--</sup>]. Practically all the phosphorus is present as phosphate. The analysis shows a slight excess of cations, but as secreted the saliva has a pH of 6.6 because of the high CO<sub>2</sub> tension in the

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mouth. After exposure to air, saliva becomes more alkaline through loss of  $\text{CO}_2$ . Saliva obtained by chewing paraffin or rubber is more alkaline than the usual saliva by about 0.1 pH.<sup>54, 109</sup> It becomes more acid during the excretion of an alkaline urine. Following the ingestion of food it becomes more alkaline for a short time. Experiments have shown no definite correlation between acidity of saliva, the content of phosphate or calcium and dental caries or tartar formation.

There is a species variation in concentration and relative composition of saliva. The saliva of the dog, for example, differs from that of man in that it has a higher  $[\text{Na}^+]$ ,  $[\text{HCO}_3^-]$  and  $[\text{Cl}^-]$ , and a lower  $[\text{K}^+]$ , and is much more viscid.

In general, composition of the saliva is not altered by diet, but does vary in the same direction as changes in the blood, in respect to calcium, phosphate, sodium and potassium. The chloride is changed but little, and  $\text{NaCl}$  injections have no effect. This is an example of the relation of the original composition of secretions to alterations in blood compositions, which has been previously mentioned (p. 60). Injected salts affect the parotid and submaxillary glands differently.<sup>11, 13</sup> The  $[\text{Na}^+]$  and  $[\text{Cl}^-]$  vary with the rate of secretion, but the  $[\text{K}^+]$  is quite constant.<sup>47</sup>

Urea is present in the saliva in approximately the same concentration as in blood. The urea may be converted in part to ammonia in the mouth, so that the preceding statement applies more correctly to the sum of the urea and ammonia.<sup>53</sup>

Thiocyanates have been reported to be normally present in saliva, especially after smoking. The salivary thiocyanate presumably is derived from traces of cyanides or thiocyanates ingested, but it has no special significance. Iodides when ingested are also found in the saliva and other fluids of the body.

### Gastric Juice

The digestive function of the gastric juice is to make the food constituents more fluid, more soluble and more nearly isotonic by the action of its  $[\text{H}^+]$ , pepsin, renin and lipase. It is gradually mixed with the food held in the fundus until the peristaltic movements of the stomach, over a period of one to five hours, carry the contents through the pylorus into the intestine.

No minerals and little water are absorbed from the stomach. The studies of Cannon<sup>20</sup> have shown that the opening and closing of the pylorus are intimately connected with the acidity of the gastric contents, but in the passage of food from the stomach the motility of the stomach is more important than the acidity.

The stomach is innervated by the splanchnic and vagus nerves; stimulation of the nerves causes secretion of juice, and section of the nerves reduces but does not abolish secretion. Stimulation of the gastric

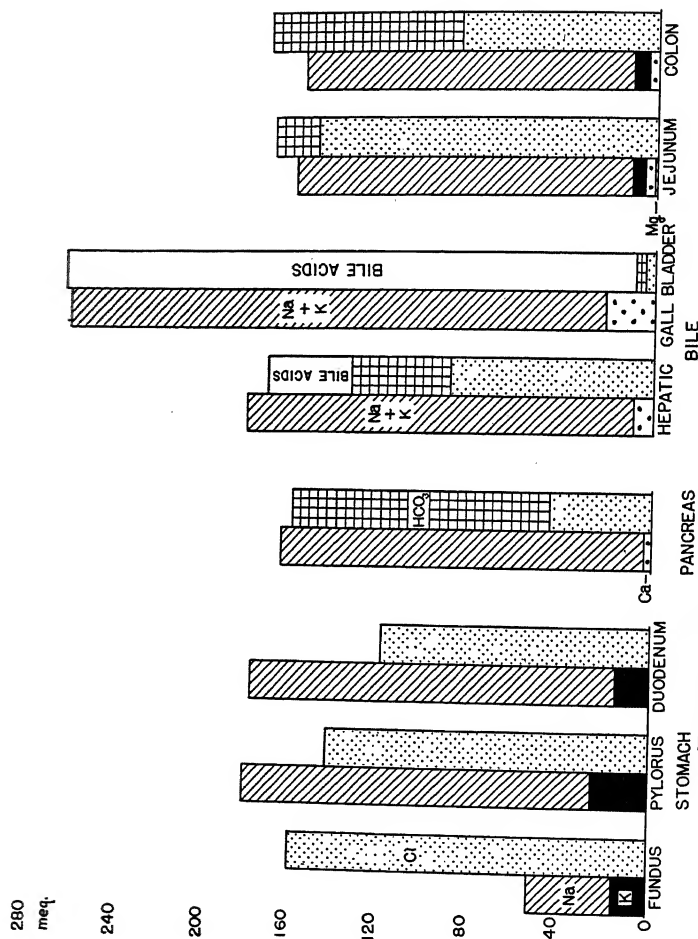


Figure 7. Composition of Alimentary Secretions.\*  
 \* Calculated from the literature.

glands produces in the cells an extractable substance which has been called gastrin. This material injected into other animals causes secretion of gastric juice. The conditions which influence the secretion in health and disease have been reviewed by Carlson.<sup>22</sup>

**Composition of gastric juice.**—The gastric juice is unique among body fluids because of its high acidity, which was recognized a century ago by Prout as due to HCl. The analysis of the minerals by Bidder and Schmidt was the first attempt to quantitate this acidity. Many studies on titration of acidity and measurement of pH have followed, for both experimental and diagnostic purposes. The earlier methods of obtaining the juice by fistula as in the classical studies of Beaumont on Alexis St. Martin, or by experimentally produced gastric pouches, devised by Heidenhain and Pavlov, or by stomach tube, all resulted in obtaining a fluid containing considerable mucus and cations. When gastric juice is obtained under stimulation of histamine, the flow is increased two to five times, and the extraneous factors are diminished to a minimum.

The specific gravity of gastric juice is about 1.007. It contains 0.5-0.6 per cent of total solids, and 0.11-0.14 per cent inorganic material.  $\Delta = -0.53$  to  $-0.58$  °C., which shows it to have the same osmotic pressure as the blood. Gamble and McIver<sup>37</sup> have shown by analysis that gastric juice is essentially isotonic with the blood.

Pure parietal secretion not only is free of mineral cations, but is an isotonic solution of  $H^+$  and  $Cl^-$ , 0.167 mM (pH 0.92). The acid secretion of the fundus is diluted with a pyloric secretion which is practically neutral and high in cations and  $[HCO_3^-]$ , along with mucous secretions and possible transudates. Hence, the slower the secretory rate, the less acid the mixed juice.<sup>24</sup> (See Figure 7.)

The  $[K^+]$  and the K/Na ratio in mixed gastric juice are higher than in blood serum. The  $[K^+]$  is the same in all parts of the stomach, and is not altered by the rate of secretion. For this reason, in addition to its  $[H^+]$  and  $[Cl^-]$  the juice is shown to be a secretion and not a transudate from the blood.<sup>6, 69</sup> Ammonium is nearly always present in small amount. The phosphorus is nearly all organic.<sup>30</sup> The juice of lower acidity has been shown by Hollander<sup>63, 64</sup> to have a higher cation concentration; the total base of the gastric juice increases as the acidity falls.

How the stomach cells secrete a juice a million times as acid as the blood remains a mystery. Attempts have been made to explain it in terms of the transformation of hypothetical organic compounds con- or another phosphorylated organic compound. We shall await with interest deductions that may be drawn from recent histochemical work taining chlorine<sup>51</sup> and also in terms of the participation of phosphoric acid. The latter might, conceivably, be formed by hydrolysis of one under way in the laboratory of Linderstrøm-Lang.

**Alterations in composition.**—Pavlov thought that different foods caused variation in amounts of the different ferments in the secretion. Changes in mineral composition depend upon alterations in the blood, and not upon food intakes.<sup>43</sup>

Gilman and Cowgill<sup>43</sup> have shown that the osmotic pressure of the gastric juice varies directly with that of the serum. The  $[\text{Cl}^-]$  of the gastric juice is equivalent to the total base of the serum water. When alterations in osmotic pressure are brought about by injection of  $\text{NaCl}$  or glucose or by water deprivation, corresponding changes in gastric juices occur. But when  $[\text{Cl}^-]$  in the juice is thus increased, it is not what is called the "free  $\text{HCl}$ " that is augmented. Changes in the acid-base equilibrium of the serum as evidenced by altered  $[\text{HCO}_3^-]$  bring about changes in the gastric juice.<sup>5, 16</sup> The stomach can never, then, secrete a juice showing "hyperacidity," but can show only "hypersecretion."

**Volume of gastric juice.**—It has been estimated that the total amount of gastric juice normally secreted in 24 hours is of the order of five liters, and thus is equivalent to nearly double the total chloride in the blood plasma. There is always a small flow of gastric juice, but during meals this is greatly augmented. It was shown by Pavlov that the amount of flow is proportional to the amount of food ingested. Twice the amount of the same kind of food calls forth twice the amount of secretion, but different foods cause different amounts.

**Alkaline tide.**—The formation of acid gastric juice even though partly balanced by the secretion of the alkaline pancreatic and intestinal juices, renders the blood more alkaline. This alkalinity is undoubtedly a factor in the alkaline tide of the urine. Hubbard *et al.*<sup>68</sup> have shown that in achlorhydria the alkaline tide is diminished or absent. That this is not the sole cause of the diminished urinary acidity is evident from the review by Brunton.<sup>18</sup> The secretion causes, in both infants and adults, a definite fall in the plasma  $[\text{Cl}^-]$  lasting for about 40 minutes, and returning to normal in about the same time. Following a meal, due to the resorption of  $\text{Cl}^-$ , extra  $\text{Cl}^-$  is found in the urine.

**Clinical variations of acidity.**—The acidity of the gastric contents in relation to clinical conditions has been much studied. About 12 per cent of normal persons, especially after middle age, show hypochlorhydria. Cancer of the stomach and pernicious anemia are associated with achlorhydria, and it has been often observed in many other diseases.<sup>91</sup> Diminished acidity has been observed after parathyroidectomy and after viosterol ingestion. Peptic ulcer is often associated with hypersecretion. At normal acidity of gastric digestion bacteria are rapidly killed and therefore the gastric juice is an important factor in preventing infections produced by eating contaminated foods.

**Clinical measurement of acidity.**—To study the secretory activity of the stomach, a test meal, consisting usually of tea and toast, is given, and the stomach contents are subsequently removed. The meal itself has an acidity of pH 5-6, and considerable buffer value, so that it requires a considerable amount of alkali to titrate it from pH 3 to some arbitrary value, such as pH 10. The gastric contents removed obviously represent the material introduced plus any saliva that may have been swallowed, plus the gastric juice secreted, and minus that material which has passed into the intestine. On this material it is customary to measure the free acidity by titration to pH 3.0, and the combined acidity by further titration to an alkaline end point with phenolphthalein or some other indicator. The "combined acidity" measures therefore only the concentration of this buffer material still remaining in the stomach; the "free acidity," the acid above that necessary to neutralize the buffer to pH 3.0. Therefore this test gives an insight into the activity of the stomach in relation to a given meal, but does not actually measure either the amount of gastric juice or of acid secreted. Under these conditions the response of a normal person gives average values of 30-50 cc. of 0.1 *N*/100 cc., or 30-50 mM of free HCl/l.

In infants on cow's milk diets, the acidity rarely is greater than pH 4.0-5.0.<sup>7, 110</sup> Peptic activity is at its maximum at pH 1.6 and ceases at 3.0, and therefore does not take place in infants.

**Effects of loss of gastric juice.**—When vomiting occurs both fluids and minerals are lost from the body. If gastric juice only is lost, the body chloride is depleted, and dehydration, alkalosis and "gastric tetany" may result. In water intoxication also, chloride is lost by vomiting. If the alkaline intestinal contents are regurgitated cations may be lost also. Presumably such is the case in the vomiting of pregnancy. The best treatment for vomiting is, therefore, the administration of salt.

Gamble and McIver<sup>36</sup> showed that following pyloric obstruction in the rabbit, an animal which is unable to vomit, the secretions into the stomach contained three times the water in the plasma, twice the cations in the plasma, and over three and a half times the total plasma chloride. This extreme and rapid removal of the body anions and to a lesser extent of the cations resulted in alkalosis and almost complete disappearance of extracellular body fluids, and death supervened in 36-48 hours.

### Pancreatic Juice

The main function of pancreatic juice relates to the enzymes which it contains. The organic matter may be more than one per cent, and it is coagulable by heat. The water and minerals continue the process of

rendering the foodstuffs fluid and of further regulating acidity and osmotic pressure.

The secretion of pancreatic juice is intermittent, and occurs when food enters the small intestine. Like the other secretory glands of the gastrointestinal system, it has a double nerve supply from the vagus and splanchnic nerves, stimulation of which increases secretion. But the main stimulation to the flow of pancreatic juice is secretin. This hormone is formed in the intestinal mucosa when the acid stomach contents pass into the intestine, and are absorbed into the blood. It functions even after section of the nerves.

Occasional analyses have been obtained on human material from fistulae, but most of our knowledge comes from experimental animals. It has long been known that the pancreatic secretion is the most alkaline in the body. The pH is approximately 8.0. Thus it neutralizes the acid material from the stomach. Gamble and McIver<sup>38</sup> have shown that the total base is of approximately the same concentration as that found in blood plasma. They state that the  $\text{Cl}^-$  forms approximately 25-50 per cent of the anions and the  $\text{HCO}_3^-$  the remainder, but Ball<sup>9</sup> has found the values to be 75 per cent and 25 per cent respectively. (See Figure 7.) The  $[\text{HCO}_3^-]$  and  $[\text{Cl}^-]$  vary inversely, but the sum of the two is constant. Ingestion of vitamin D causes diminution of  $[\text{Cl}^-]$  and increase in  $[\text{HCO}_3^-]$ .<sup>10</sup> The alkalinity of the pancreatic juice increases with increased secretion.

Injectations of acid and alkali cause changes in the composition of the pancreatic juice with respect to the  $[\text{HCO}_3^-]$ , in the same direction but to a lesser extent than in the blood serum.<sup>8</sup> Elevations of  $[\text{Na}^+]$  and  $[\text{K}^+]$  in the serum are reflected in the juice, but those of  $[\text{Ca}^{++}]$ ,  $[\text{H}_2\text{PO}_4^-]$ ,  $[\text{HPO}_4^-]$ , and  $[\text{Mg}^{++}]$  only to a limited extent.

The loss of juice through pancreatic fistula was studied in a dog by Gamble and McIver.<sup>39</sup> The dog was fed an adequate diet of meat washed to remove sodium and chloride. Loss of 21 per cent of the body weight ensued, and death resulted in 14 days. The analyses of the fluid removed showed the loss was due to depletion of extracellular body water.<sup>72</sup> The loss of body fluid resulted in dehydration. If this loss were from interstitial fluids alone it would represent their complete removal. But they must have supplied most of it because the other possible source of these ions, the blood serum, was not sufficiently lowered in volume to show concentration, as measured by plasma protein, until after the tenth day. This is an excellent illustration of the tenacity with which the blood serum remains constant in composition at the expense of the other body fluids. The mineral composition of the plasma also remained constant until the tenth day. At this point the loss of  $\text{Na}^+$  from the plasma could no longer be replaced and severe acidosis supervened.

## Bile and Liver

**Bile.**—Bile, the external secretion of the liver, contains, among other ingredients, bile salts and bile pigments, which are important in digestion of fat and in pigment metabolism, respectively. Bile is secreted more or less constantly, and stored and concentrated in the gall bladder, from which it is intermittently ejected into the intestine, especially during the process of digestion. The interest in its mineral content is of recent origin.

The composition of bile varies more widely than the other secretions, both in different individuals and in the same individual at different times. It is partly related to the rate of secretion, though the factors which regulate secretion rate are not well-defined. In most studies the bile has been obtained from fistulae, during operations, and from experimental animals.

The mineral content of hepatic bile is practically the same as that of blood plasma<sup>38</sup> (see Figure 7). The  $[\text{HCO}_3^-]$  is about twice that found in plasma, and the  $[\text{Cl}^-]$  is correspondingly less. Other observers have reported that the bile acids are present to the extent of about 40 meq., with a corresponding further decrease in the  $[\text{Cl}^-]$ . Apparently this factor is variable. The total base may be greater than in serum, but because of the formation of salts with bile acids, and the unknown degree of their ionization, the osmotic pressure as measured by the freezing point is practically the same as that of serum. The pH is 7.1-8.6.

The gall bladder secretes a milky white mucous fluid free from bile salts and containing calcium and magnesium in approximately the same concentration as serum.<sup>70</sup>

When bile is stored in the gall bladder changes take place.<sup>97</sup> The concentration of solids is about three per cent in liver bile and 16 per cent in gall-bladder bile. Water,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  are reabsorbed so that most of the anions remaining are the bile acids, which increase to six times their previous concentration. Cations are also reabsorbed, but not to so great an extent; their total concentration is increased about 50 per cent. Calcium is increased in concentration four- or five-fold, although it is probably reabsorbed to some extent.<sup>86</sup> The determination of osmotic pressure by the freezing point method gives values for gall-bladder bile which are the same as for hepatic bile and blood serum. The pH is 6.3-7.0. Certain foreign substances are secreted in the liver bile, concentrated in the gall bladder, and ultimately eliminated in the feces. Tetraiodophenolphthalein is thus eliminated and because this dye is relatively impermeable to the x-rays, it enables roentgenologic detection of the outline of the gall bladder and the presence of gall stones.

Bile aids in the emulsification of fats and the absorption of fatty



acid ions. Further, in the presence of bile, calcium and magnesium soaps are rendered more soluble and are better absorbed from the intestine, perhaps through the effect upon the increase of calcium ionization.<sup>14</sup> When bile is excluded from the intestine by fistula, vitamin D is not effective unless given subcutaneously; hence under these conditions rickets and negative calcium balances occur even with ample vitamin D in the diet.<sup>46, 59, 118</sup>

Gall stones may contain over 95 per cent of cholesterol, or considerable amounts of the pigment, bilirubin. They often occur as a result of infection. They contain also Ca, PO<sub>4</sub>, CO<sub>3</sub>, Na and K.<sup>93</sup> Magnesium and bile salts prevent precipitation of cholesterol. Increased intakes of magnesium are not reflected in the bile.

When bile passages are obstructed and the bile cannot be emptied normally, it escapes into the blood stream and jaundice results. The clotting of the blood is diminished and serious hemorrhages occur. The state of the serum calcium is not definitely altered.<sup>48</sup> In long-standing obstruction the bones become osteoporotic. Cantarow<sup>21</sup> states that calcium therapy empirically is of great benefit in both obstructive jaundice and toxic liver necrosis.

The relation of bile to fat, pigment and cholesterol metabolism lies beyond the scope of this discussion.

**Liver.**—The liver is important not only in the physiology of the proximate principals but also in water and mineral metabolism. The large amount of material brought to it by the intestine can be disposed of by formation of bile or by passage either to the blood stream or to the lymph. The important studies of Beckmann<sup>12</sup> have indicated that it may act as a reservoir for withholding excess amounts of the minerals brought to it, and then gradually eliminating them by one of its three paths.

Large amounts of sodium introduced through the portal vein are excreted principally in the bile and lymph, potassium principally in the blood, and calcium almost entirely in the blood. Chloride is slowly excreted into the blood stream, bicarbonate into the bile, and phosphate principally into the blood and lymph, and less into the bile. Sulfates are retained in the liver. It is here that ethereal sulfates are formed, and phenols thus detoxified.

Fiske and SubbaRow<sup>33</sup> state that one-third of the total acid-soluble phosphorus of the liver is  $\alpha$ -glycerophosphate. The phosphorus compounds with adenine and creatine are related to those in the muscle. Changes in these occur during both exercise and fever, but none of significance in sodium, potassium or calcium.<sup>19, 67</sup>

The liver also functions as a storehouse for iron and copper, and is important in the metabolism of these elements.

### Intestinal Juices

The intestinal juices supply enzymes which continue the splitting of the food material entering the gut. It takes the food about four to six hours to traverse the small intestine, during which time active absorption of nutrients takes place, as has been described on page 83. The residue then passes into the colon where it remains from 6 to 24 hours, or in the condition of constipation, for days. Here occurs the final stage of disintegration of food material, partly by digestive juices and partly by bacteria. The remaining absorption, especially of water and of  $\text{Na}^+$  and  $\text{Cl}^-$ , and excretion of calcium, magnesium and phosphate takes place.

Very little is known about the physiology of the intestinal juice, primarily because of the difficulty of obtaining it. The available evidence has been summarized by Brugsch<sup>17</sup> and Heupke.<sup>58</sup> The amount of intestinal juice is large, between two and three liters per day, and variable, according to the demands of digestion. Secretion in the colon is slower than elsewhere in the intestine. The fluids are essentially transudates of the blood plasma, as shown by the freezing point,  $\Delta = -0.60^\circ\text{C}$ . The pH has been given as varying between 6.5 and 8.0, and the ash as 0.9 per cent. The few analyses that have been made (on dogs) indicate that it is primarily a solution of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ . The composition of the secretions of the duodenum, jejunum and colon are given in Figure 7.

The juices from various parts of the intestine show no great divergence in the pattern of the cations, but the farther down the tract the greater is the proportion of  $[\text{HCO}_3^-]$  to the  $[\text{Cl}^-]$ . In the jejunum the value of the  $[\text{HCO}_3^-]$  is about 20 meq., in the ileum, 80 meq., and in the colon 90 meq. These data were obtained by analyses of material in intestinal loops. Removal of the secretion leads to dehydration.<sup>55</sup>

Injection of minerals into the blood stream is reflected in the composition of the intestinal juices as in other secretions. When a hypertonic solution of  $\text{Na}^+$  and  $\text{Cl}^-$  is injected, the  $[\text{Na}^+]$  is increased, accompanied in the upper portion by a preponderance of  $\text{Cl}^-$  and in the lower segment by greater  $[\text{HCO}_3^-]$ .

The studies of Goldschmidt and Dayton<sup>45</sup> which have been described on page 84, do not indicate primarily the nature of the intestinal secretion, but only the alteration of the fluids which had been placed in the gut so that they might be absorbed.

### EXTERNAL SECRETIONS

#### Milk

Mammary glands are present in both sexes and are rudimentary until puberty. Their development comes only with sexual maturity of the ovaries and is associated with the ripening of the corpus luteum.

The secretion of milk is normally initiated by the birth of the young. Extracts of the uterine wall injected into virgin animals cause a secretion of milk. Extracts of the fetus inhibit the flow of milk. One of the active substances of the anterior hypophysis, called prolactin, also stimulates the flow of milk. Nervous stimuli and emotional states affect the rate of secretion, and decreased secretion may often be traced to this source. But the main stimulus to milk secretion is associated with the actual removal of milk. When it is no longer removed from the breast, secretion diminishes and ceases after a few days. The greater the amount of stimulus, especially by suckling, the greater is the milk supply. The amount secreted is normally about one liter per day, but in some women volumes of three liters are not uncommon. Under constant stimulation milk secretion may be continued for a very long time, up to three or four years, as is the custom in Japan.

The dairy cow has been developed and selected for its capacity to secrete large amounts of milk, and therefore presents the problem of the stress of lactation in its most extreme form, both in relation to the rate of secretion and to the extension of the periods of lactation.

There is a wealth of material concerning the mineral composition of milks.<sup>102</sup> Bunge enunciated the theory that the mineral composition of the milk parallels that of the fetus. This was soon abandoned and replaced by the thesis that the composition of the milk is related to the time required for the young to double its birth weight; the more rapid the growth the greater is the concentration of minerals in the milk. The data of Abderhalden are well known, and are given in Table 9. The

Table 9.—Relation of Mineral Elements of Milk to Rate of Growth.\*

Species	Days Required to Double Birth Weight	Composition of Milk			
		Protein (%)	Ash (%)	Calcium (%)	Phosphorus (%)
Human .....	180	1.6	0.2	0.02	0.02
Horse .....	60	2.0	0.4	0.09	0.06
Cow .....	47	3.5	0.7	0.12	0.09
Goat .....	22	3.7	0.78	0.14	0.18
Sheep .....	15	4.9	0.84	0.18	0.11
Swine .....	14	5.2	0.80	0.18	0.14
Dog .....	9	7.4	1.33	0.32	0.22
Rabbit .....	6	14.4	2.50	0.65	0.43

\* Abderhalden.<sup>1</sup>

interest which this once aroused has passed, but it led to many investigations of the mineral composition of both the milk and the new-born of many species.

A comparison of woman's, cow's and rat's milk is shown in Table 10. The milks of various species differ in the water content so that the milk

Table 10.—Comparison of the Composition of Rat's, Cow's and Woman's Milk.\*

	Rat's Milk		Cow's Milk		Woman's Milk	
	(gm./l.)	(meq./l.)	(gm./l.)	(meq./l.)	(gm./l.)	(meq./l.)
Sodium .....	0.76	33	0.61	26.5	0.11	5.0
Potassium .....	1.70	43	1.54	39.5	0.48	12.2
Calcium .....	3.49	174	1.22	61.0	0.34	17.0
Magnesium .....	0.31	26	0.13	11.0	0.05	4.0
Total cationogens .....		296		138.0		38.2
Chloride .....	1.17	33	1.16	32.8	0.36	10.0
Phosphorus .....	2.72	158 †	0.90	52.0 †	0.15	8.7 †
Sulfur ‡ .....			0.31	19.4	0.036	2.2
Total anionogens .....		191		104.2		20.9
Iron .....	0.007		0.0007		0.002	
Copper .....	0.007		0.0006		0.0005	
	(%)		(%)		(%)	
Protein .....	11.77		3.42		1.25	
Carbohydrate .....	2.83		4.75		7.50	
Fat .....	14.79		3.50		3.50	
Ash .....	1.50		0.75		0.20	
Total solids (sum) .....	30.89		12.42		12.45	
Calories, as protein .....	24.6		21.7		7.5	
Calories, as fat .....	69.5		48.8		47.4	
Calories, as carbohydrate..	5.9		29.5		45.1	
Ca/P ratio .....	1.28		1.35		2.26	
pH .....	6.6		6.7		7.0	
Ash/100 gm. solids .....	4.8		6.0		1.6	
Ash/100 gm. protein .....	12.8		22.0		16.0	
Calories/liter .....	195		660		680	
Ash/100 Calories .....	0.75		1.1		0.3	

\* Cox and Mueller.<sup>26</sup> Table 3. Reproduced by permission of the *Journal of Nutrition*.

† mM × 1.8.

‡ Not included in the original.

as secreted apparently differs very greatly in the amounts of the minerals found. It is therefore more satisfactory to consider milk either in terms of total calories or in terms of dried material.<sup>94</sup> On this basis milks of various species are not so widely divergent. The best correlation is between the ash and the protein. On the other hand they cannot be made alike by any method of calculation, as they vary according to species, in protein, fat and carbohydrate, and in the relation of solutes and of individual minerals to each other, both absolutely and relatively. Analytical data, when used with the ordinary assumptions, make it appear that milks are hypotonic, but the freezing points, which reflect directly the activity of water, show that cow's milk is isotonic with the blood.

Although rat's milk shows great divergence from cow's milk because

of the low carbohydrate and high fat, the mineral composition much more closely resembles that of the other species. The points of interest are high calcium and phosphorus, which correlate well with high protein content, and the relatively great amounts of iron and copper.

Compared to cow's milk, woman's milk is approximately only one-fourth as concentrated in minerals, but is actually richer in iron and relatively richer in potassium, which is in excess of the sodium. The high proportion of calcium and phosphorus, with the Ca/P ratio of 1.3/1 in cow's milk and 2.3/1 in woman's milk, makes this a unique secretion and also food. The pH is 6.6-7.0. The preponderance of mineral cations over anions is also unusual in foods, but not in secretion's. In woman's milk approximately  $\frac{1}{2}$  of the phosphorus is in inorganic form, and  $\frac{1}{4}$  is diffusible; in cow's milk  $\frac{2}{3}$  is inorganic and  $\frac{1}{3}$  is diffusible. Of the calcium in woman's milk  $\frac{1}{2}$  is diffusible, and in cow's milk  $\frac{1}{3}$ .<sup>56</sup> Half of the calcium in cow's milk is present as caseinate. The problem of the states of the calcium and phosphorus is therefore a very difficult one. The proportional amounts of calcium, phosphorus and magnesium are the same in the milk of all species, and this is comparable to the uniformity of these minerals in the bone of all species.<sup>120</sup> Cow's milk normally contains about 0.1 per cent citrate. The fats of milk are synthesized in the mammary gland from the blood phospholipids,<sup>85</sup> and are therefore related to phosphorus metabolism.<sup>121</sup>

Variations in the composition of milk occur in early and late lactation. Colostrum contains slightly more of all the minerals, and the  $[\text{Na}^+]$ ,  $[\text{K}^+]$  and  $[\text{Cl}^-]$  are twice as great as in milk.<sup>66</sup> In general only the organic factors in the milk can be made to fluctuate widely due to dietary influence. When given large doses of vitamin D, cows and women secrete a milk of high vitamin D potency.<sup>57, 75</sup> Iron, copper and manganese in the diet have no effect on milk, but iodine is excreted in the milk (and perhaps arsenic). Minerals in the milk secreted are not constant,<sup>34</sup> but under ordinary conditions they fluctuate within small limits, and the factors which cause these changes have not been fully evaluated.<sup>15, 88, 89</sup> The chief effect of restriction of protein, fat and carbohydrate, but especially of protein, is to diminish the amount of milk secreted.<sup>85</sup> The amount of minerals secreted is large, and causes a great drain on the mother.<sup>104</sup> The effect of milk secretion on the maternal organism is considered under *Pregnancy and Lactation* (p. 340), and the importance of milk as a food in Chapters 13 and 14.

## EXCRETIONS

### Urine

**Mechanism of urine secretion.**—The modern studies on the physiology of the kidney form a brilliant chapter, and have greatly clarified the problem of excretion in general. The question as to whether excre-

tion is a filtration, according to the thesis of Ludwig, or a secretion, as maintained by Heidenhain, has led to many studies epitomized by the work of Cushny, Richards, Marshall, Rehberg and others, and summarized by Peters<sup>92</sup> and by Smith.<sup>105</sup> The modern viewpoint is that the renal mechanism involves essentially five structures: the glomerulus, the proximal convoluted tubule, the thin part of Henle's loop, the distal convoluted tubule, and the collecting tubule. From a comparative study of the kidneys of many species, Marshall<sup>83</sup> has recently been able to reassess the function of these various structures.

According to the modern consensus, the processes of urine formation may be summarized as filtration by the glomerulus, subsequent reabsorption in the tubule, and tubular secretion. Only in those animals having a thin part in the loop of Henle is the urine concentration greater than that of the plasma. There is convincing evidence that secretion takes place in the proximal convoluted tubule. If the glomerulus secretes, it does so to a much lesser extent. All filtrable materials such as glucose and chloride are excreted by the glomerulus, and may be absent from the urine of animals having no glomeruli. The convoluted tubule reabsorbs not only water, but also such substances as glucose and chloride. The convoluted tubule may also secrete similar substances and may maintain a secretion pressure higher than the blood pressure. This theory accounts for the so-called threshold substances which are present in the blood, but are not necessarily found in the urine.

Starling<sup>108</sup> has propounded the theory that the portion of the blood which does not permeate the vessel walls exerts an osmotic pressure which, balanced against the pressure in the vessels, regulates urine secretion. As the osmotic pressure of the proteins falls, the secretion rate increases. This oncotic pressure is about 20-25 mm. of mercury out of a total pressure of 5000.

**Factors affecting urine secretion.**—The volume of urine secreted depends upon the composition of the blood and nervous and hormonal control. In addition, the amount of secretion is increased by increase of the surface of the capillary bed, the permeability of the capillaries and the blood pressure; and is decreased by increase of the oncotic pressure and the hydrostatic pressure in the convoluted tubule.<sup>125</sup> Stated in another way, diminished blood pressure or increased oncotic pressure and hydrostatic pressure in the tubule will increase reabsorption. Increased permeability may permit the passage of protein into the urine. Reduction in the blood volume may increase the viscosity and diminish the amount of circulation to the point where the normal oxidation and nutrition of the cells of the tubules is impaired. This may result in altered secretory power. Conditions which affect the degree

of hydration of the cells may also influence the tubules. It has been suggested that swelling of the cells of the tubules may cause anuria.

The kidney is innervated by the splanchnic nerves. Stimulation results in cessation of flow of urine, and section of these nerves removes inhibition. These vasomotor constrictors may be influenced from the medulla. Cerebral control is strongly indicated not only from "salt puncture," but also from the association of migraine headaches, epilepsy and hysteria with polyuria. That the pituitary gland exerts an influence upon renal flow is manifest in diabetes insipidus.

The kidney has great reserve capacity. Only part of the glomeruli or the capillaries in them are patent at one time during normal function. More than 50 per cent of kidney substance can be removed and the remainder yet be adequate. The character of the blood coming to the kidney is probably the determining factor in the secretion of urine. This is evidenced in the formulas for kidney function, such as those of Ambard<sup>3</sup> or those used in the modern "clearance" studies, which relate the volume and composition of the urine to that of the blood.

**Clearance.**—Clearance is expressed in terms of the amount of blood from which a given material has been completely removed in the formation of the urine in a given time. The amount which the kidney "clears" may be used as a measure of kidney function. It is calculated from the determined concentration of the substance in the blood and that in the urine, and from the amount of urine formed.

This type of procedure was developed by Rehberg to study the excretion of organic solutes, such as creatinine, on the assumption that they were excreted by the glomerulus and not reabsorbed by the tubule. They could thus be used as a measure of the amount of glomerular filtration and the amount of resorption of water by the tubule. This subject has been discussed at length by Peters<sup>92</sup> and by Smith<sup>105</sup> and is alluded to here only to point the argument<sup>†</sup> that the amount of a particular substance in the urine depends upon its concentration in the blood. In the excretion of a given substance, its concentration in the urine and the volume of the urine must be considered as well as the concentration in the blood.

**Volume of urine.**—*Normal volumes.*—The amount of urine excreted depends, in the last analysis, upon the amount of water ingested (see Chapter 12, *Water Metabolism*). Under ordinary conditions an infant produces from 450 to 600 cc. of urine per day, and an adult from 800 to 1500 cc. The urine flow varies between 0.5 cc. and 20 cc. per minute. The daily urine contains 50-60 gm. of solids, of which 20-25 gm. are minerals. This is accomplished by the secretion of a very large amount of ultrafiltrate, of which over 90 per cent is resorbed into the blood. In mammals the amount of glomerular filtrate has been estimated at 100-300 cc./kg./hr.<sup>73</sup> In man this calculates to 100-150 l./day, or over 40

times the plasma volume. It is therefore easy to calculate that it is necessary for the tubules to reabsorb nearly 150 l. of water and the equivalent of 900 gm. of NaCl, and 325 gm. of  $\text{NaHCO}_3$  per day. Thus the accuracy with which the water excretion is controlled is evident, for one per cent less water resorption would double the amount of urine formed.

*Alteration of volume.*—Ingestion of very concentrated NaCl solution, 1.65 per cent, leads to diminished urine flow, and may result in temporary edema. Large salt intakes in the food, irrespective of the water intake, may also lead to  $\text{Na}^+$ ,  $\text{Cl}^-$  and water retention in normal persons, but especially in infants and in patients with nephrotic edema. In general, concentrated urines are acid and dilute urines less so. Alkalosis resulting from hyperventilation causes a diuresis, and acidosis resulting from breathing  $\text{CO}_2$  causes diminished urine flow. On the other hand, ingestion of mineral acids or of organic acids which are not oxidized causes removal of mineral cations from body fluids, and leads to increase in the volume of urine. Conversely, alkali ingestion diminishes water excretion. When the skin is chilled a condition called cold diuresis is induced.

When any constituent of the blood plasma is increased, the excess is quickly excreted in the urine. Thus ingested or injected  $\text{K}^+$  and  $\text{Cl}^-$  in isotonic solution are rapidly excreted. When ingested or injected intravenously, physiological saline acts as only a mild diuretic, because  $\text{Na}^+$  and  $\text{Cl}^-$  are rapidly distributed to the tissues. Injected hypertonic saline solutions act as strong diuretics. But the  $[\text{Na}^+]$  and  $[\text{Cl}^-]$  in the plasma are not the only factors determining their secretion, for the formation of urine is certainly connected with the colloid pressure in the blood. Thus when serum is replaced by an equal amount of physiological saline diuresis takes place.

Volume of urine further depends upon the acid-base equilibrium of the blood. Andrews<sup>4</sup> showed that when the  $\text{CO}_2$  capacity of the blood was below 45 vol. per cent, hypertonic solutions in huge amounts had no diuretic effect. When the blood is alkalinized by the injection of bicarbonate, large diuresis occurs. Substances such as hypertonic glucose which, when given intravenously, increase the osmotic pressure act as strong diuretics.

Cushny has differentiated between glomerular and tubular diuretics. Salt and sugar are in the first class, sulfate, nitrate and phosphate in the second. The latter substances, when ingested, prevent absorption in the intestine and cause catharsis, and when injected interfere with reabsorption and cause diuresis.

*Maximal volumes.*—The volume of urine which can be excreted is amazing. Haldane and Priestley,<sup>49, 95</sup> after drinking 5.5 l. of water in six hours, noted a secretion of 1200 cc./hr. Over 20 l. of urine per day



may be excreted by diabetics. In diabetes insipidus reports of extreme urine outputs, up to 43 l./day, have been made. This is equivalent to the whole of the body water. These large volumes were the result of large water intakes and the urine was very dilute. But urine outputs may be associated with concentrated urine. In experimental animals with high salt or high urea intakes Gamble *et al.*<sup>40</sup> have been able to increase the daily urine output to more than half the body weight, with urines of nearly maximal concentration.

The volume of the urine depends upon the amount of water to be excreted. Ordinarily the amount of water available for excretion is greater than the necessary minimum. However, the water itself may be a limiting factor, for the kidney has limited capacity to concentrate the constituents in the urine.

**Concentration of urine.**—The normal concentration of the minerals in urine is dependent upon the average mineral and water intakes. It may thus be higher or lower than that of the blood. The electrolytes of average urine represent, according to Hammarsten, about 560 mosM/l., or about 1.6 times the concentration in the blood plasma, independent of the urea, which represents a fraction equally large; and the excretion per day of  $\text{Na}^+$  and  $\text{Cl}^-$  is the equivalent of 15 gm. of NaCl. According to Clark's data (Table 39, p. 333) an average daily output is equivalent to only 6 gm. of NaCl. If a volume of 1000 cc. is assumed, this concentration of minerals is only 280 mosM/l., but if the volume is 1400 cc. the concentration is 200 mosM/l.—equal to 85 and 60 per cent respectively, of the concentration of the blood plasma.

Gamble *et al.*<sup>40</sup> have studied kidney function by the addition to the food of large amounts of either salts or urea or mixtures of these, so as to obtain the effects of each practically alone or in combination. The water intake was not limited. NaCl additions resulted in only 1.2 osmolar urine. Mixtures of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  in any proportion gave the same values as NaCl alone. But the kidney which excretes urine of maximal concentration with respect to phosphates and urea may still excrete chloride without increase in volume of urine. Thus mixtures of salts and urea cause production of urine of a higher osmotic pressure than either alone.

The maximal concentrations of rat's urine observed by Gamble, as measured by the freezing point, equalled 2.1 osM/l., or seven times the osmotic pressure of the blood. This value was found when the animals were excreting salts and urea at a slow rate. Adolph<sup>2</sup> by means of urea intake, and Gamble, with urea and NaCl, have shown that the concentration of the urine depends upon the rate of excretion. The more rapidly salt or urea is removed from the body, the more water accompanies it. With a fivefold increase in the rate of urea excretion the concentration

drops one-half; under similar circumstances with the excretion of  $\text{Na}^+$  and  $\text{Cl}^-$  the concentration of the urine drops one-fourth.

The power of the kidney to concentrate is different for the different minerals. According to Cushny,<sup>27</sup> in normal urine  $\text{Na}^+$  is concentrated to an amount equal to that in the blood plasma,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{Cl}^-$  to 2 times that in the plasma,  $\text{K}^+$  to 7,  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^-$  to 30,  $\text{NH}_4^+$  to 40, and  $\text{SO}_4^{--}$  to 60 times.

**Composition of urine.**—In the course of the flux of minerals in the body, alterations in the composition of the blood occur, and minerals are thus offered for excretion. Alterations in cellular components must first be communicated to the interstitial fluids, and thence to the blood before the kidney can act. This is not necessarily accomplished in a short time, but may take days. Ingested water is rapidly excreted. An equal amount of an isotonic solution containing  $\text{Na}^+$  and  $\text{Cl}^-$  is only gradually eliminated, but an isotonic solution of  $\text{K}^+$  and  $\text{Cl}^-$  is excreted as rapidly as water. The reason is that in the first and last cases the composition of the serum is altered, but  $\text{Na}^+$  and  $\text{Cl}^-$  are transferred to the interstitial fluid so rapidly that only small changes in the serum volume or composition occur. The removal of these substances in the urine is so adjusted as to preserve the normal concentrations in the body with remarkable constancy. This is true not only of the ions collectively, but of each ion individually.

The kidney is the principal organ of the body for the excretion of water, and of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{--}$ , though not of the  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$ , nor of the greater amount of the phosphate ions. The  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  are the main mineral components of the fluids in the body, and the kidney has been called the organ which maintains the integrity of the body fluids. The excretion of the kidney thus not only regulates the osmotic and ionic equilibria, but also plays a principal role in governing the acid-base economy.

Perhaps the best way to see how the kidney accomplishes its work is to consider the composition of normal urine. The average mineral composition of the urine is given in Table 39 (p. 333). But average values have little meaning, for urine varies from individual to individual and from day to day. An example of this variation is given in Table 11 and Figure 8. These data represent the urine formed by a normal individual with average salt intake, and also with low  $\text{NaCl}$  intake, and with additions of  $\text{KCl}$  and  $\text{NH}_4\text{Cl}$  to the same basal diet. The amounts of each mineral found in the urine of normal men in equilibrium are determined by the intake of each.

**Acidity of the urine.**—Because the average diet is nearly neutral and body metabolism produces excess anions, and the cations are partly excreted by the bowel, there usually remains an excess of anions to be excreted in the urine. When excess alkali is ingested the kidney readily

Table 11.—Mineral Excretion in Urine and Feces as Affected by Intake.\*

	Low Salt	KCl† Addition	NaCl‡ Addition	NH <sub>4</sub> Cl + NaCl§ Addition
Urine				
Volume (cc.)	1920	2135	1960	2470
pH	6.2	6.4	6.5	5.4
Titratable acid (meq.)	23.7	23.9	22.0	51.5
NH <sub>4</sub> (meq.)	31.8	37.7	29.5	111.8
K (meq.)	58.2	195.5	59.0	112.5
Na and Mg. by difference (meq.)	16.5	44.0	73.5	152.5
Ca (meq.)	13.7	16.8	15.5	27.2
Total positive minerals (meq.)	88.4	256.3	148.0	292.2
Cl (meq.)	9.7	171.0	68.2	323.0
P (gm.)	0.84	0.81	0.85	1.26
HPO <sub>4</sub> and H <sub>2</sub> PO <sub>4</sub> as P (meq.) <sup>1</sup>	32.5	34.0	35.5	41.8
SO <sub>4</sub> (meq.)	36.6	37.0	36.3	37.9
Organic acid (meq.)	48.2	47.8	49.2	60.1
Total N (gm.)	10.2	10.6	10.7	13.7
Creatinine (gm.)	1.67	1.72	1.53	1.69
Feces				
Dry weight (gm.)	22.0	22.8	25.2	19.6
K (meq.)	11.5	14.8	16.7	11.5
Na and Mg. by difference (meq.)	13.1	14.7	15.4	11.3
Ca (meq.)	17.7	17.6	19.4	16.5
Total positive minerals (meq.)	42.3	47.1	51.5	39.3
Cl (meq.)	0.26	0.64	1.30	0.82
P (gm.)	0.35	0.37	0.41	0.29
PO <sub>4</sub> (meq.) <sup>2</sup>	34.0	35.8	39.6	28.0
Total N (gm.)	1.39	1.49	1.70	1.26
Intake				
Fluid (cc.)	3485	3464	3506	3473
K (meq.)	81.9	208.6	77.3	75.0
Na and Mg. by difference (meq.)	41.2	46.1	163.9	164.9
Ca (meq.)	22.6	22.1	22.9	23.1
Total positive minerals (meq.)	145.7	276.8	264.1	263.0
Cl (meq.)	18.7	152.6	139.2	364.3
P (gm.)	1.16	1.18	1.36	1.23
PO <sub>4</sub> (meq.) <sup>3</sup>	67.6	68.6	79.0	71.4
Total N (gm.)	12.3	12.3	13.4	12.0
Calories	2645	2645	2645	2645

\* These data represent sample daily values taken from Table 3 of Loeb *et al.*,<sup>70</sup> a study of a normal individual for 45 consecutive days on a constant diet except for salt additions.

† KCl addition = 120 mM.

‡ NaCl addition = 134 mM.

§ NaCl addition = 134 mM, NH<sub>4</sub>Cl addition = 224 mM.

<sup>1</sup> Calculated as 1.2, 1.3, 1.3, 1.0 eq./M.

<sup>2</sup> Calculated as 3.0 eq./M.

<sup>3</sup> Calculated as 1.8 eq./M.

In balance studies phosphorus should be calculated as having uniform value in intake, urine and feces. See Table 39, page 333.

excretes an alkaline urine. The cations in the urine consist of  $\text{NH}_4^+$ , which is formed from urea in the kidney, and the mineral cations,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ . The mineral anions are  $\text{Cl}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{--}$ ,  $\text{SO}_4^{--}$ ; and the non-mineral anions are  $\text{HCO}_3^-$  and the organic anions. There are present also undissociated acids. The body conserves the mineral

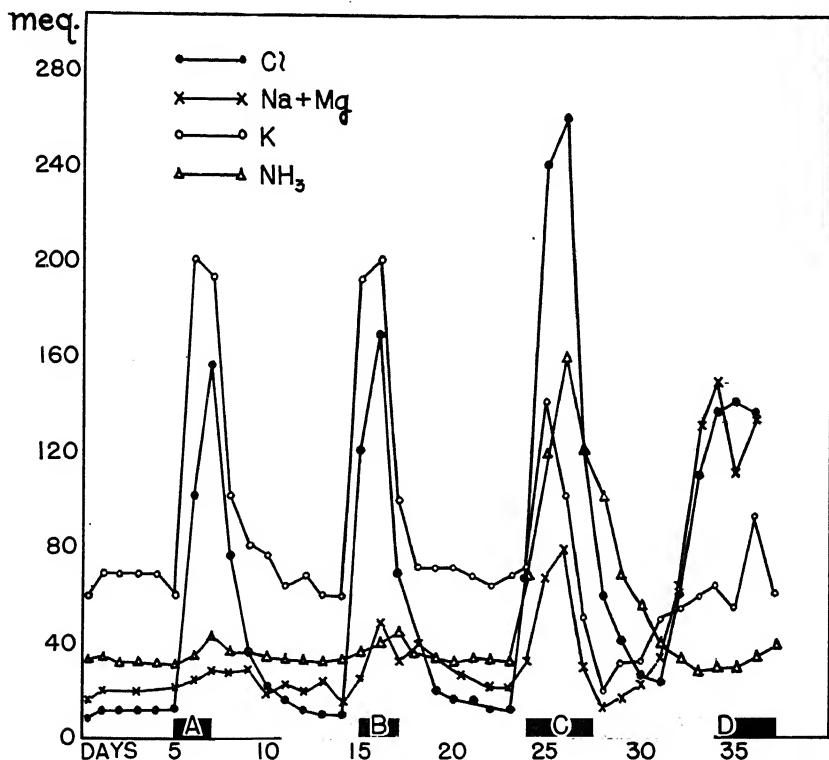


FIGURE 8. Mineral Excretion in Urine and Feces as Affected by Intake.\*

\* Loeb *et al.*,<sup>76</sup> Figure 2. Reproduced by permission of the *Journal of Clinical Investigation*. This graph represents the mineral excretion of a normal individual on a constant basal diet with the following salt additions: A and B = 120 mM of KCl per day; C = 224 mM of  $\text{NH}_4\text{Cl}$  per day; D = 134 mM of NaCl per day. See also Table 11 and text.

cations by the production of urine with an excess of mineral anions. Its acidity is pH 4.8-7.8, average, 6.0. The mechanism by which the body eliminates excesses of either mineral anions or cations with a minimum expenditure of the other is described in Chapter 13.

*Sodium and chloride.*—Under most circumstances  $\text{Na}^+$  and  $\text{Cl}^-$  occur in the urine in approximately equivalent amounts. This is largely

because of the large amount of NaCl in the diet. (See Table 11 and Figure 8.) Also, when edema fluid is being dissipated the  $\text{Na}^+$  and  $\text{Cl}^-$  are excreted in nearly equal amounts, because that is their proportion in the interstitial fluid.

When no NaCl is given, or during fasting, the urine excreted is practically devoid of both  $\text{Na}^+$  and  $\text{Cl}^-$ . The small amount present is due to depletion of intracellular constituents and with it the accompanying intercellular fluid. When  $\text{Na}^+$  and  $\text{Cl}^-$  are lost in sweat (see p. 92) or by way of the bowel (see p. 338) less remains to be excreted in the urine. Under these circumstances the urine may become almost free of  $\text{Na}^+$  and  $\text{Cl}^-$ .

*Sodium or chloride.*—In conditions in which there has been a loss of  $\text{Cl}^-$ , as in loss of gastric secretion, when this  $\text{Cl}^-$  is supplied as NaCl the body retains practically all of the  $\text{Cl}^-$  and excretes the  $\text{Na}^+$  in conjunction with  $\text{HCO}_3^-$ . When HCl is fed, the  $\text{Na}^+$  content of the body is depleted by being excreted with the  $\text{Cl}^-$ . Feeding of NaCl in this case causes excretion of  $\text{Cl}^-$  and retention of  $\text{Na}^+$ . When  $\text{Cl}^-$  is taken independently, as above, or when  $\text{CaCl}_2$  or  $\text{NH}_4\text{Cl}$  is given, the  $\text{Na}^+$  elimination is less than it would be if it were not for the formation of  $\text{NH}_4^+$ . (See Table 11 and Figure 8.) However, some positive minerals are drawn upon. These come in part from the blood plasma, body fluids and bones, and in addition there is a shift of calcium, magnesium and phosphate from the feces to the urine. When  $\text{NaHCO}_3$  is fed, both  $\text{Na}^+$  and  $\text{HCO}_3^-$  in the urine are increased. When  $\text{HCO}_3^-$  is thus increased, the  $\text{NH}_4^+$  is diminished, and  $\text{Cl}^-$  and  $\text{SO}_4^{--}$  are not necessarily increased.

*Potassium.*—Aside from the excess ingested the  $\text{K}^+$  in the urine comes from cellular catabolism.  $\text{K}^+$  is not stored in the intercellular fluid, but is rapidly excreted by the kidney. When KCl is ingested both  $\text{K}^+$  and  $\text{Cl}^-$  are excreted simultaneously in the urine (see Table 11 and Figure 8).

*Calcium and magnesium.*—Nearly all the excreted calcium and magnesium are found in the feces. Therefore, except as they may provide extra cations under the stress of anion excretion, the kidney does not regulate their removal.

*Phosphate.*—Although the phosphates play an important part in anion excretion, the urine is not the principal means of their elimination. (See Chapters 7, 13, 14.) Still smaller proportions of calcium, magnesium and phosphate are found in the urine of infants than of adults.

*Sulfate.*—The sulfur excretion comes primarily from the catabolism of proteins, and therefore continues as long as life exists. When extra sulfates are fed, either as neutral or as acid-producing salts, they appear almost wholly in the urine. (See Chapter 8, *Sulfur*.)

**Summary.**—This simplified account of kidney function has not given details of its action under many physiological and pathological condi-

tions which have been studied in great detail. High or low atmospheric pressures, forced ventilation, excessive breathing or breathing air of different compositions, sweating, diarrhea, fistula, infections, intoxications, altered circulation and kidney disease all affect the process of urine excretion. Moreover, to present the function of the minerals alone apart from the rest of the body economy gives a very incomplete picture. The reader must therefore be referred to the specialized literature on the kidney.

It may be stated in summary that excretion in the kidney depends primarily upon the water and salts ingested. In its regulation of the body water under stress, so far as its capacity lies, the kidney maintains the exact structure and concentration of the blood plasma. It is more sensitive to disturbances of composition of the plasma than to those of plasma volume or even intercellular fluid volume. When the normal concentrations of individual ions cannot be maintained the osmotic relations are guarded by the substitution of other ions ( $\text{HCO}_3^-$  for  $\text{Cl}^-$ , or *vice versa*). Thus the integrity of the total base is maintained with greater tenacity than that of the individual anions or even cations. In extreme cases fluctuations of osmotic pressure occur. But the blood is thus altered only after readjustment of the components throughout the body fluids has failed.

The material excreted by the kidney is dependent not only upon the intake, but also upon the materials simultaneously excreted by the skin and bowel. Excretion of water by the kidney is fundamental, but excretion of calcium, magnesium and phosphate by the bowel is just as essential. Unlike the kidney, the intestine is hardly ever at fault through inability to excrete the necessary materials, but rather through failure to retain adequate amounts when the supply is limited. When the bowel becomes active as an excretory organ, as will be shown in the next section, it may deprive the urine of nearly all its sodium and chloride. Under conditions requiring production of sweat, these ions are lost through the skin and the urine is correspondingly depleted. Thus the mineral excretion of either skin or bowel may outweigh that of the kidney. This emphasizes the fact that the kidney has the capacity not only to excrete soluble minerals but also to conserve them.

## Feces

**Absorption and excretion in the intestine.**—The intestinal contents are acted upon by the organic and mineral constituents of the gastrointestinal juices as well as by the enzymes. The protein, fat and carbohydrate are split, and in addition the minerals with which they are associated are freed. To realize the active part that minerals play in absorption from the intestine, consider only the example of fat metabolism. The formation of soaps, the emulsification of fats with bile

acids and pancreatic juice, and the formation of phospholipids are prerequisite to the utilization of fats. A review of the physiology of intestinal absorption has been given by Verzář.<sup>122</sup>

Although absorption of some drugs may take place in the mouth and even from the mucous surfaces of the nose, eye, and lacrimal duct, from the viewpoint of mineral and water metabolism, no important absorption occurs here or even in the stomach. The main absorption takes place from the small intestine. The material from the intestine is absorbed partly through the portal system and partly through the lacteals and thoracic duct into the general circulation. This transfer is accomplished by the action of cells on particulate matter, or by the absorption of lipoid-soluble materials at their surface, or by the interchange of fluids in the intercellular spaces. The muscular activities of the intestine and the fibres associated with the villi are supposed to aid absorption mechanically through pressure changes. Intra-abdominal pressure has also been given as a factor in this pumping action.

With regard to absorption, as to secretion, the same controversies have raged between vitalists and mechanists. An excellent historical and theoretical review has been given by Goldschmidt.<sup>44</sup> The studies of the mechanisms have recently been reviewed by Höber.<sup>81</sup>

Hyper-, hypo- and isotonic salt solutions introduced into the intestinal tract are all absorbed into the blood stream. Before absorption they become more nearly isotonic with the blood plasma. Hypertonic solutions lose electrolytes to the blood at the same time that they receive water from it, and for hypotonic solutions the reverse is true. As material is moved down the intestine and the solutions become more isotonic, the reabsorption of the fluids and minerals progresses. When water is introduced into the intestine it is mixed with the secretions which are formed until the concentration of  $\text{Na}^+$  and  $\text{Cl}^-$ , for example, is equivalent to 0.16 per cent NaCl. Optimal absorption of  $\text{Na}^+$  and  $\text{Cl}^-$  is found with solutions of 0.6 per cent NaCl. From 1.0 to 1.5 per cent of NaCl, the absorption rate diminishes progressively. In still higher concentration, absorption, apparently from increased diffusion, again takes place. Such solutions may injure the intestinal mucosa. Thus the process of intestinal absorption is a complicated one, regulated by the interdependent absorption and secretion of water and electrolytes. The mechanism is similar to that in the kidney and elsewhere in the body. The extent of control by the nervous system is unknown.<sup>41, 42</sup>

When solutions of single salts are placed in the intestine, other ions are secreted into the lumen. From various mixtures and concentrations of  $\text{Na}^+$  and  $\text{K}^+$  or of  $\text{K}^+$  and  $\text{Ca}^{++}$ , or more complicated mixtures, unequal portions of each are absorbed. In general the ease of absorption follows the Hofmeister series. Phosphate and sulfate are absorbed with difficulty, and chloride is readily absorbed. Magnesium and calcium are

less well absorbed than sodium and potassium. It is therefore easy to understand why  $\text{MgSO}_4$  should act as a cathartic.

The absorption of such materials as glucose has been studied in isolated loops and the whole body.<sup>25</sup> In intestinal loops, although hypertonic solutions are diluted with intestinal juices, the rate of absorption is proportional to the concentration, and becomes slower as absorption progresses.<sup>96</sup> The amounts of electrolytes present influence the rate of absorption.<sup>78</sup> In the intact animal the secretion of gastric juice is so regulated that the material presenting itself for absorption in the jejunum is of nearly constant concentration.

The acidity of the small intestine in its upper portion is pH 5.9-6.6, in the middle 6.2-7.3.<sup>100</sup> The inorganic matter of the intestinal contents must consist of the anions and cations of strong acids and their slightly soluble salts, or a mixture of weak acids and their anions, in proportion to the pH of the intestinal tract. Acid ingestion or an acid reaction in the intestine increases absorption and alkali diminishes it.

The part played by the large intestine in the active excretion and reabsorption of minerals in normal digestion is hardly known at all. Goldschmidt and Dayton,<sup>45</sup> who studied the isolated large intestine of dogs, were the first to establish clearly that transfers of water and ions into the large intestine, and in the reverse direction, actually take place. Although calcium and phosphate can be absorbed here, it is well known that this is the principal site of their normal excretion. Here too most of the remainder of the fluid is reabsorbed, and the residue becomes feces.

**Composition of feces.**—The feces are a composite of: (1) unabsorbed material foreign to the body, which never entered the body economy; (2) the residuum of gastrointestinal secretions; (3) salts excreted by the intestine; (4) products of bacterial metabolism.

The amount of feces passed is variable in the same individual and from one individual to another. In starvation little if any feces are excreted. A normal amount may be placed at 50-300 gm./day, of which the water content represents an average of 80 per cent. The solid matter equals 15-150 gm., with an average of about 40 gm. Of this one-fourth to one-half represents the bodies of living and dead bacteria. The feces normally contain about 1.5 gm. of nitrogen per day; about half of this is bacterial nitrogen, and the remainder is so unrelated to the amount in the food ingested, and its protein nature is so doubtful, that it probably represents a secretion of the intestine. In rare cases specific undigested proteins may be recovered. The feces contain practically no  $\text{NH}_4^+$  or carbohydrate (except fiber).

The minerals in the feces of the normal adult consist mostly of calcium, magnesium and phosphate, constituting 88, 69 and 43 per cent respectively of the excretion of these substances (see Tables 39 and 40, pp. 333 and 336. The calcium and magnesium are present both as soaps



and as phosphates. The feces contain less than five per cent of the excreted sodium and chloride. The potassium excreted by this route has more than twice the combining value of the sodium, although the amount in the food is usually only one-third. The fecal sulfur constitutes one-fifth of the sulfur excretion. Sulfur has received very little attention, and the forms in which it occurs in the feces are not known. The excretion of iron is almost wholly by the bowel, even when injected parenterally.

In the feces of infants, either breast-fed or artificially fed, similar or even larger proportions of calcium, magnesium and phosphate are found. The sodium and chloride are variable, as in the adults'; and because the infants' stools are more fluid (see Table 12) or because they remain for a shorter time in the intestinal tract, a slightly larger proportion of these elements is found here.

Table 12.—Composition of the Feces of Infants.\*

	Normal stools <sup>1</sup>		Average per infant per day		Very loose stools <sup>3</sup>	
	(gm.)	(meq.)	Loose stools <sup>2</sup> (gm.)	(meq.)	(gm.)	(meq.)
Total .....	44.55		145.95		314.16	
Water (cc.) .....	35.6		133.00		293.00	
Dry matter .....	8.75		12.95		21.16	
Protein .....	2.26		3.56		5.56	
Total fat .....	3.12		4.04		7.97	
Neutral fat .....	1.09		2.83		5.70	
Free fatty acids .....	0.34		0.72		2.44	
Soap fats .....	1.69	6.8	0.50	2.0	0.74	3.0
Cl .....	0.022	0.6	0.14	4.0	0.28	8.0
P (mM × 3) .....	0.28	27.0	0.31	30.0	0.26	25.0
Total anionogens .....		34.4		36.0		36.0
Na .....	0.030	1.3	0.126	5.5	0.27	11.6
K .....	0.15	3.8	0.30	7.7	0.68	17.5
Ca .....	0.73	36.5	0.78	39.0	0.59	30.0
Mg .....	0.057	4.8	0.060	5.0	0.072	6.0
Total cationogens .....		46.4		57.2		65.1
Excess cationogens .....		12.0		21.2		29.1

\* Calculated from Table 3 of Holt, Courtney and Fales.<sup>65</sup>

<sup>1</sup> Seven patients, eleven periods.

<sup>2</sup> Eight patients, fourteen periods.

<sup>3</sup> Six patients, ten periods.

*Relation of minerals to fluid content.*—Laviertes, in Peters' laboratory, calculated the minerals in the feces on the basis of the water content, in terms of mM/kg. of water.<sup>92</sup> He found the  $[Na^+ + K^+]$  of this fluid to be (in 70 per cent of the cases) 110-167 meq./kg. of water. The  $[Cl^-]$  varied between 17 and 164 meq./kg. of water. The  $[K^+]$  in nearly all cases exceeded the  $[Na^+]$ , which may be due in part to its presence in cellular material. It is difficult to determine how much of the cal-

cium, magnesium, phosphate and carbonate were present in the solid or liquid phase. How these varied with the relation of solid to fluid, or with acid-base equilibrium, is not detailed. The values for the positive and negative equivalents were neither parallel nor inverse. However, this novel viewpoint may prove very fruitful, for in some cases these analyses show that the osmotic pressure in the fluid of feces does not differ markedly from that of other body fluids, and may closely resemble an ultrafiltrate of the blood.

*Fats in the feces.*—Normally the fat content of the feces is equivalent to 2-10 per cent of the ingested fat, or about 15 per cent of the dried matter in the feces. This fat, in normal stools, has been shown to vary so widely from the dietary fat as to be in the nature of a secretion of the intestine.<sup>106</sup> A large portion is found in the bacteria.<sup>107</sup> F. Müller found that on a milk diet this fat consisted of 20-27 per cent neutral fat, 40-50 per cent free fatty acids, and 20-40 per cent soaps. In addition volatile fatty acids are found in small proportions. Some fat is excreted even when none is ingested. Large additions of fat to the diet do not materially affect the percentage of intake lost. Telfer<sup>119</sup> has shown that, in infants, the larger the amount of fat ingested, the greater the amount of calcium found as soaps, and the less the fecal phosphate. Diseases of the pancreas and exclusion of bile from the intestinal tract lead to great losses of neutral fat (although this is not conclusive evidence of abnormality of the pancreas). In diseases where the absorption is interfered with, as sprue and celiac disease, the proportions may be normal but the loss may amount to 80-90 per cent of the intake. In acute diarrhea also, large amounts of normally split fat and soaps are lost<sup>123</sup> (see Table 12).

*Acidity of the feces.*—At birth the meconium has a pH of 6.1.<sup>90</sup> The normal feces are alkaline. On an average mixed diet the pH is 8.0-9.0.<sup>77</sup> Similar values are obtained in infants fed cow's milk mixtures; with breast milk or malt soup pH 4.5-5.2 is found. The question of the factors which govern fecal acidity is of interest. Neither acid nor alkaline diets, nor even the presence of acidosis has any effect on the pH or buffer value of the feces.<sup>74</sup> Hence titration and pH determination do not give an indication of the acid-base equilibrium of the body. Lactose and ketogenic diets have a very slight acidifying effect.<sup>101</sup> The bacterial flora vary. It has been suggested that the type of bacteria determines the acidity because of their particular type of fermentative end products. But ingestion of organic acids such as lactic acid has no effect on fecal pH. It is more probable that the acidity of the intestinal contents influences the type of flora, for the acidity largely determines the optimal environment. After starvation, regardless of the previous reaction, Ylppö<sup>126</sup> found the pH to be 6.4.

The main chemical influences upon the pH are the fatty acids and

soaps, and the carbonates and phosphates. This complicated buffer system is regulated primarily by the calcium and fatty acids. When the fatty acid is excessive, acid stools result. If calcium is increased, the alkalinity increases. It is probable that the changes in acidity of the feces which have been used in quantitating vitamin D are thus explained. The change in the pH of feces of rats from 7.4 on a rickets-producing diet to 6.2-6.4 after cod-liver oil may be interpreted as due to reduction of calcium in the feces.<sup>71, 127</sup> A similar alteration has not been observed in children.<sup>98</sup>

**Factors affecting composition.**—The feces are the main regulator of calcium and phosphorus economy. It is not profitable to discuss the alterations of the feces without consideration of the effects of intakes of calcium and phosphorus in relation to their utilization and retention. This subject is discussed in Chapter 14. It will suffice to state here that when larger amounts of calcium, phosphorus and magnesium are ingested, greater amounts of each are found in the feces. The excretion depends upon the relative as well as the absolute amounts of each ingested. The amount present in the feces does not represent merely unabsorbed insoluble products, but actual excretion into the gut after absorption. Even on calcium-low or calcium-free diets large amounts of calcium may be excreted in the feces. Intravenous or parenteral calcium or phosphate is also excreted partly by the lower ileum, and especially by the large bowel.

The excess of positive or negative minerals in the food affects the excretion of calcium and phosphate. With acid diets urinary calcium and phosphate are increased, and on alkaline diets the fecal excretion of both is increased. Large amounts of acid intake draw upon the calcium and phosphorus stored in the bones, and thus increase the excretion of positive mineral elements.

The effect of the endocrine glands, especially the thyroid and parathyroid, upon fecal excretion is mentioned in the next chapter (p. 114). Vitamin D, in the curing of rickets, diminishes the fecal excretion of phosphate and calcium slightly, and increases the urinary calcium and especially phosphate.

**Reduction in the intestine.**—The intestinal tract, probably because of the action of bacteria, forms a medium in which substances are reduced; for example, iron salts before their absorption are reduced from ferric to ferrous compounds, and sulfur to  $H_2S$ .

**Diarrhea.**—Diarrhea may lead to large losses of minerals by the bowel. In this case the  $Na^+$  and  $Cl^-$  are increased more than the other ions. Dehydration and acidosis of serious or fatal proportions may result, as in cholera or intestinal intoxication (cholera infantum).<sup>60, 126</sup> The mineral composition of normal and loose, but not diarrheal, stools of infants has been studied by Holt, Courtney and Fales,<sup>65</sup> and is given

in Table 12. Their data show that, compared to the normal, there is practically no increase in phosphate in the loose stools. The water increased more than 8 times. The dry matter increased 2.5 times; this is accounted for largely by the fat, which increased 2.5 times. The neutral fat increased 5 times, the fatty acids 7 times, and the soaps diminished to one-half their usual value. The chloride increased 14 times, sodium 9 and potassium 4 times. The increase in chloride, sodium and potassium was accompanied by a diminished excretion of these in the urine, but was sufficient to cause negative balances of sodium and potassium in the cases with very loose stools. Strangely, the chloride is so diminished in the urine as to allow increased positive balances by computation, and thus casts considerable question on this chloride value. This shows, in a unique experiment, the inverse relation of these materials in the urine and feces. A computation of the positive and negative mineral equivalents, although sulfur values are absent, shows an increasing preponderance of cations over anions as the stools become looser. This type of mineral loss was called "relative acidosis" by Steinitz.

**Constipation and catharsis.**—Diarrhea is characterized by increase of water in the intestinal contents and increased motility of the intestine; constipation results from inspissation and stasis. Ordinarily increased water intake is readily absorbed, and causes no increase in the fecal elimination. But excessive ingestion, as in water intoxication, may be sufficiently great to cause diarrhea.

The water content of the feces is increased when there is a higher content of hydrophilic colloids. Such materials (agar-agar, bran, psylla) increase the bulk of the feces, not only by the materials present, but by the water which they retain. Because the motility of the intestine is stimulated by increased mass, these substances have been used to regulate constipation. They act further because the more rapidly the intestinal contents are moved along the tract the less absorption takes place, and hence the more fluid the feces. Diarrhea is often a result of intestinal irritation and hence increased motility. Cathartics such as castor oil or croton oil have this effect. Water excretion into the intestinal tract or failure of reabsorption lead to passage of feces of higher water content.<sup>99</sup> Magnesium and sodium sulfate have long been used as cathartics because they are not absorbed, but draw water into the intestine. Evacuation of a large amount of watery feces may take place within two hours after ingestion, in contrast to the usual slow passage of food materials in 12-24 hours.

### Sweat

The main function of sweat in the animal economy is to facilitate heat loss by evaporation. This regulation of body temperature will be

considered in Chapter 12, *Water Metabolism*. The temperature and humidity of the environment are the main external factors, and work is the principal physiological cause of sweating. It occurs also as a result of fevers produced by the activity of microorganisms, or of dehydration such as is caused by the injection of hypertonic solutions. Great interest has been shown recently in the production of artificial fevers by diathermy and radio-thermy in the control of disease. The purpose of such procedures may be either the increased temperature attained, or the profuse sweating which results.

**Mechanism of secretion.**—The regulation of heat dissipation is effected by the central nervous system through the heat center, either centrally or by reflex action. Heating the blood going to the brain causes sweating. The stimulus is transmitted by secretory nerves running in the sympathetic nervous system, and may be independent of vasomotor control. If one sciatic nerve of a dog is cut and the animal is heated, the paw of the leg whose nerve is gone remains dry, while the other three sweat. Raising the temperature of the body 0.5-1 °C. stimulates the activity of the sweat glands. Excretion of sweat may be restricted to a single area; when one arm is heated it may sweat and the rest of the body remain dry. There is also a local mechanism of control, for sweating takes place when pilocarpine is administered after the section of all nerves. Atropine inhibits sweating. An important part of heat regulation is the calorogenic function of the thyroid; in hyperfunction excessive sweating is a prominent feature; in hypofunction the reverse.

The heat regulation mechanism is poorly developed in premature and new-born infants. So, too, their ability to excrete sweat is limited.

The exact nature of sweat formation is unknown. The concentration of minerals in the subcutaneous tissue is of undoubted importance, whether the sweat glands draw from this source directly or indirectly through the blood. The problem of sweat is of interest from many viewpoints—energy metabolism, protein metabolism, tropical hygiene, and the physiology of work and fever.

**Volume of sweat.**—In temperate climates the main regulator of sweat is the amount of work done. Below an environmental temperature of 25 °C. water excretion in sweat may be absent, but at higher temperatures it may exceed that in breath or urine, or both. The volume of sweat may exceed 10 l./day. Fishberg and Bierman<sup>32</sup> found that persons heated to 41 °C. with radiothermy produced 3-4 liters of sweat in three hours. Similar amounts of sweat, or even greater—up to 2.5 l./hour—were found by Moss<sup>37</sup> in miners.

**Composition of sweat.**—Sweat from the axilla and pubic region, called apocrine sweat, is neutral or alkaline, and contains oily odoriferous secretions; that from the rest of the body is called exocrine sweat.

It is the latter which can be greatly increased. Sweat from the face, body and hands may differ in composition. Work sweat and heat sweat differ in that the former is more acid.<sup>113</sup> Little has been reported on the "acid sweat" of rheumatism and other diseases. Normal values are of the order of pH 4.0-5.5.<sup>79</sup>

Sweat contains no special or unique constituents other than the oily secretions. Hence, contrary to the popular belief, inhibition of the excretion does not lead to death unless through inability to dissipate heat. There is a rare clinical condition of absence of the sweat glands. These persons exhibit no abnormalities except a rise in body temperature as the result of mild exercise.

Besides the minerals, sweat contains urea, uric acid, amino-acids, ammonium, sugar and lactic acid. The urea content of sweat may be so high in nephritic subjects with uremia that crystals of urea may be scraped off the skin after evaporation of the sweat. The lactic acid content, normally 75-300 mg./100 cc., has been reported as high as 1750 mg./100 cc. during a Marathon run. Because of this capacity to secrete lactic acid in concentrations greater than that in the blood it is evident that sweat is a true secretion and not a transudate.

Those factors which affect the blood composition also cause alteration in sweat. Considerable differences in the mineral composition of sweat are reported, but regardless of its variation it is always hypotonic. The components are the same as those found in other secretions, but in different proportions. The calcium, magnesium and potassium occur in the same concentrations as in plasma. The  $[Na^+]$  and  $[Cl^-]$  are nearly equal, but are less than in blood.<sup>50, 124</sup> Various authors report values for  $[Na^+]$  and  $[Cl^-]$  as 15-140 meq./l. In most laboratory experiments values of 50-100 meq. are reported. Low salt diets cause a diminution of the  $[Na^+]$  and  $[Cl^-]$  in sweat. The mineral composition of sweat is given below.\*

Composition of Sweat, Expressed in Meq./l. <sup>82</sup>							
Water lost (cc.)	Hours	$[Na^+]$	$[K^+]$	$[Ca^{++}]$	$[Cl^-]$	Lactic acid	pH
	1	82	5.1	5.7	85	25.1	4.10
4020	3	83	4.9	5.6	83	25.2	4.05

Moss has reported sweat of similar composition in miners. In their study of the reaction to prolonged dry heat, Dill *et al.*<sup>31</sup> state that, although they have obtained similar values for short periods after active exercise, the continued excretion of sweat of such high concentrations is unthinkable. Their values for  $[Na^+]$  and  $[Cl^-]$  averaged 15 meq./l. for 3-7 liters of sweat, equivalent to a total of only 7 gm. of NaCl. Certainly values ten times as high are not reasonable. In the tropics

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the amount of salt in sweat must diminish after acclimatization. Thus, as with urinary secretion, the composition can be altered to suit the necessity for excretion.

When the  $[Na^+]$  is decreased the  $[K^+]$  is raised, but only to a slight extent. In the experiments cited the  $[K^+]$  equalled one-half the  $[Na^+]$ . In some cases potassium may be nearly absent. Swanson and Iob<sup>112</sup> have indicated that in infants, who have a comparatively great potassium intake, the  $[K^+]$  may exceed the  $[Na^+]$  in sweat, and that failure to take account of this excretion may lead to serious errors in considering the potassium balances of infants. Sulfates occur principally as esters. Phosphates are very small in amount.

**Excessive sweating.**—Excessive sweating may reduce the amount of urine and render it almost chloride free. Sweating reduces the  $[HCO_3^-]$  of the blood, and also the alveolar  $CO_2$  tension.<sup>80</sup> It causes concentration of the blood<sup>23</sup> with diminished  $[Cl^-]$  and [total base].<sup>114</sup> This dehydration results in heat prostration. If water alone is ingested diminished osmotic pressure of the blood results, and heat cramps may follow.<sup>115, 117</sup> Relief from the symptoms of excessive sweating is afforded by ingestion of NaCl, especially with water, whereas drinking water alone causes further loss of  $Na^+$  and  $Cl^-$  in the sweat.<sup>62, 81, 82</sup> When physiological saline solution (0.9 per cent NaCl) is administered it is retained in the tissues with such avidity that it may act as an antidiuretic.<sup>28</sup>

For a long time dry or moist heat, and more recently electrical heating, have been used as methods of inducing profuse sweating to aid a failing kidney. By the elimination of large amounts of material, especially  $Na^+$ ,  $Cl^-$  and urea, the skin can thus function as an important organ of excretion.

## CHANGES IN VOLUME AND COMPOSITION OF BODY FLUIDS

### Normal Cyclic Alterations

The exposition of the structure of the body in Chapter 2 showed that the mineral composition is remarkably constant. The differently constituted separate organs and fluids resist change not only in the proportions of minerals in each, but in the relation of each to the total. The constancy of the whole is therefore attained by correlation of heterogeneous parts. This condition of constancy applies to the body only in a state of physiological rest. Physiological activity causes alterations which involve the flow of minerals within the body. Even under basal conditions of complete body inactivity, anabolic and catabolic processes are in progress. Oxygen consumption and  $CO_2$  production involve the transport and exchange of minerals. The wear and tear of the renewal of cells and cellular material involve mineral transfer and loss by excretion.

The mineral interchanges are greatly increased with the performance

of additional physiological functions, either normally or under stress. In the course of the normal daily cycle the mineral changes are transient; the body systems return to the *status quo*. But disease may bring about alterations in the chemical structure of the fluids and organs. Under these conditions physiological mechanisms perform as best they may, with the help of compensatory regulation. Ultimately the changes in structure may be so great that the material for the necessary fluxes is no longer available, and life cannot continue.

*Work.*—There are two main classes of physiological activity, the performance of work and the utilization of food. Work both consumes energy and produces heat. The effects of work show primarily in  $\text{CO}_2$  production, and only secondarily in mineral metabolism. As a result of energy metabolism the products elaborated by the cells must all be secreted by the cells or absorbed into the blood stream and thus transported to the sites of excretion. The conveyance of  $\text{CO}_2$  is accomplished largely by hemoglobin, and the interplay of minerals involved is largely between the blood cells and serum. Heat dissipation is aided by the secretion of sweat; this may bring about a very considerable loss of  $\text{Na}^+$  and  $\text{Cl}^-$ , which are withdrawn from the interstitial fluids and must be replaced. This relation of sweat to mineral economy was detailed earlier in the chapter. It may serve as a single case to illustrate the very general law which is a main thesis of this book—that the movement and metabolism of the minerals and water take place in relation to each other.

The more the work the greater must become the other factor which causes interchange of water and minerals, namely the consumption and digestion of food.

*Digestion of food.*—Food must be digested, absorbed, assimilated, and the end products excreted. These activities are associated with extremely large cyclic transfers of fluids and minerals. This mechanism is analogous to a whirlpool which remains in a river in spite of the inflow and outflow, and alters its pattern only slightly whether in flood or drought. The food is changed by the gastrointestinal secretions into a solution of materials of the same osmotic pressure as the blood. This large volume of fluid is absorbed into the blood, and thence is rapidly transferred to various parts of the body. It accumulates in the interstitial spaces, organs and storage depots. After the body fluids have been thus increased they are reduced to their original volume either by excretion or by supplying materials for another digestive cycle.

The nature of this internal circulation of the minerals was evident to Forster, who said, half a century ago:

“When salt-free or salt-poor foodstuffs are transferred from the intestinal canal into the blood, they combine in fact, as is evident from my calculations, with the free salts held in the blood



which originate from destroyed body material. These are retained in the body, and transferred hereafter for further utilization. We have here a salt circulation which is comparable to the movement of an endless chain."<sup>35</sup>

**Volumes of secretions per day.**—The great flux of minerals apart from their circulation in blood and lymph is evidenced by the amounts of secretions into the gastrointestinal tract. The volumes produced in 24 hours are as follows:

Secretion	Liters
Saliva .....	1.0- 1.5
Gastric juice .....	3.0- 5.0
Bile .....	0.5- 1.0
Pancreatic juice .....	0.8- 1.0
Intestinal juices .....	2.0- 3.0
Total .....	7.3-11.5

These figures err on the low side, because most were obtained by fistulae, which tend to limit the amount secreted. Further, no one knows how much these volumes may be increased under physiological or pathological stress. However, the amount of the total daily secretion, if estimated at only 10 liters, equals three times the plasma volume, or nearly the whole of the interstitial fluid volume.

Large as these volumes are they become insignificant when compared to the amount of the ultrafiltrate of the blood plasma produced by the glomeruli of the kidney. It is well known that not all the glomeruli operate at any one time, nor are all the capillaries within a single tuft simultaneously patent; therefore the usual volumes of ultrafiltrate are not maximal. It has been conservatively estimated that only one per cent of the amount filtered appears as urine; therefore the 1-1.5 l. of urine produced in 24 hours represent 100-150 l. of this filtrate.

The magnitude of the mineral exchanges is proportional to the food intake because first, larger amounts of food call forth the secretion of more gastrointestinal juices, and secondly, larger amounts of food introduce more salts which require absorption and excretion.

**Intercellular fluid changes.**—In all these transfers the blood maintains a relatively fixed composition and volume, whereas the other body fluids, although relatively constant in composition, fluctuate in total amounts. The blood is therefore poorly adapted to the study of fluid and mineral fluxes, and information must be sought from the changes in other fluids. These are shown best in the extracellular fluid, which constitutes a reservoir containing 80 per cent of the  $\text{Na}^+$  and  $\text{Cl}^-$  and 30 per cent of the water of the whole body (see pp. 45-46). On the one hand it supplies the basic materials for secretion, and on the other it receives materials from both cells and blood. Unfortunately techniques for studying these

changes are only beginning to be developed. The amount of interstitial fluid and its mineral composition as determined by analysis of tissues have been found to vary considerably. This inconstancy is doubtless due to variability in experimental conditions. The specific relations to the cyclic transfers have not been evaluated, except grossly. Balance studies have been utilized to demonstrate changes in the body fluids over a long period of time, but not the changes of the normal digestive cycle.

**Inner circulation of individual minerals.**—So far we have discussed alterations in the body fluids mainly in relation to  $\text{Na}^+$  and  $\text{Cl}^-$ . Other ions must however be considered. In studying the minerals separately perhaps too much attention has been given to positive and negative balances, and not enough to their behavior within the body. The body shows a surprising capacity to rearrange and adjust the materials available. Minerals may be "borrowed" for use in various parts of the body and later returned to their original sites.

**Cyclic reactions of phosphorus.**—Without presenting the extensive data which have been amassed in recent advances in muscle physiology, it is sufficient to state that many cyclic reactions of the phosphorus compounds are fundamental in the contraction of muscle. A long chain of intracellular reactions depends upon the transfer of phosphorus. But outside the cells also there is a phosphorus cycle, for the digestion and utilization of fats is accomplished partly through the formation and splitting of phospholipids; and similarly, important phases of carbohydrate metabolism are carried on through the formation and hydrolysis of phosphate esters. Even without adequate phosphorus intake these mechanisms need not cease to function, for there is an almost inexhaustible supply of phosphate in the bones which may be drawn upon.

**Storage of iron.**—The ability of the body to conserve and economize minerals through intermediary processes is excellently illustrated in the case of iron. When iron intake exceeds immediate needs the excess is stored in the depots of the body, especially the liver and spleen. When new blood is formed this is available for hemoglobin formation; when blood is destroyed the iron is not lost to the body, but is redeposited in the storage depots until it is needed.

**Alterations in bone structure.**—The bones, which we often regard as a rigid framework, are capable of alterations in minerals. During pregnancy and lactation their stores of calcium and phosphate are drawn upon to provide the demands of the growing body. Interchanges within the bones themselves may occur in conditions of stress, as exemplified by prematurity. The premature infant, because of his large needs and limited intake ordinarily does not have sufficient calcium and phosphorus for bone development. Under intensive vitamin D therapy he is enabled to utilize what material he has more efficiently, so that calcium and phosphate are absorbed from the shaft of the bone and deposited at the grow-

ing ends. This process may take place in experimental animals to the extent that rickets may be healed even when balances of calcium and phosphorus are negative.

Even in the normal adult a rearrangement of bone salts is constantly taking place. By means of radium poisoning Aub and Calhoun\* were able to show the deposition of radium in the newly formed trabeculae. This is freed when calcium is mobilized. Through the course of years this radium is dissolved and redeposited until it becomes equally distributed throughout the bone. This well describes the constant movement of calcium and phosphate. Under stress the calcium and phosphate which normally flow from blood to bone may be mobilized in the reverse direction; for example, under the influence of excess parathyroid hormone.

These changes which take place in the bony structure can be effected only through the intermediary action of the body fluids.

### Pathological Variations

**Volume of extracellular fluid.**—The changes which take place in the extra- and intracellular water may be changes in volume, in composition, or both. We shall enumerate first the causes of alteration in the volume of extracellular fluid.

Among the pathological conditions affecting this portion of body water the loss of secretions is most marked. Loss of secretions occurs principally by vomiting or diarrhea or through fistula. As can be seen in Figure 7, the secretions vary in their composition, but all contain  $\text{Na}^+$  and  $\text{Cl}^-$ , which are also the main mineral constituents of extracellular fluid. If the secretions are lost from the body and their minerals not replaced, the extracellular body fluid is diminished.

Dehydration is a condition in which body fluid, *i. e.*, water and its contained minerals, is reduced. Losses of extracellular fluid in some of the acute conditions of dehydration may amount to more than two-thirds of the entire amount. The loss of fluid through fistula as a cause of dehydration has been exemplified on page 68. Both vomiting and diarrhea result in dehydration. In the former case alkalosis ensues, because more mineral anions than cations are lost, and in the latter case, acidosis follows, because more mineral cations than anions are lost (see p. 89). Loss of gastric juice is the only condition in which dehydration is associated with alkalosis, for alkalosis usually causes excess fluid to be retained, and acidosis causes dehydration (see p. 67). Thus there is a double relationship between the acid-base equilibrium and the state of body water.

The relation between certain pathological conditions and changes in body fluids may be obscure. For instance, although hitherto unsuspected,

\* Unpublished data of Drs. J. C. Aub and K. A. Calhoun.

it has recently been shown that attacks of gout are ushered in by loss of  $\text{Na}^+$  and  $\text{Cl}^-$  and that recovery is accompanied by retention.<sup>116</sup>

The effects on extracellular fluid of excessive or too little excretion are easily illustrated. Excessive loss of sweat, because it is a solution containing mainly  $\text{Na}^+$  and  $\text{Cl}^-$ , causes dehydration. Subsequent ingestion of water alone results in dilution of body fluids, and leads to heat cramps, or miner's cramps. Only water plus  $\text{NaCl}$  will relieve this condition. Diuresis also may lead to loss of interstitial fluid. If kidney damage results in loss of ability to remove electrolytes, the intercellular fluid is increased and forms edema. Administration of  $\text{NaCl}$  intensifies such a condition. In edema the intercellular fluid volume may be more than doubled.

In other pathological conditions as much as 2 liters of fluid may be stored in the pleural cavity, and 5 liters or more in the abdominal cavity.

**Interrelation of inter- and intracellular fluids.**—So far we have discussed only changes in amount of extracellular fluid without regard to changes in its composition or its effect on the cell fluid. It is known that cells are impermeable to cations, and although blood cells permit the transfer of  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , muscle cells are probably impervious to  $\text{Cl}^-$ .<sup>52</sup> Darrow and Yannet<sup>29</sup> showed, when the osmolar concentration of extracellular fluid was either raised by intraperitoneal injection of hypertonic solutions of  $\text{NaCl}$  or lowered by isotonic glucose, that an adjustment took place between the inter- and intracellular water so that the osmotic pressure between the two was equalized. This was brought about by exchange of water only. Because osmotic pressure is maintained equally throughout the body, added  $\text{NaCl}$  raises the osmotic pressure in all compartments.

A usual salt consumption per day is 11-12 gm. of  $\text{NaCl}$ , or 200 mM, which is equivalent to 1200 cc. of an isosmolal solution. This is equal to 10 per cent of the total volume of the interstitial fluid. Hypothetical cases are given below to show the different effects on the extra- and intracellular fluids of the addition and removal of this arbitrary amount of salt in isosmolal solution (examples 1 and 2) and of the addition and removal of the same amount of salt without water (examples 3 and 4). The third example is a simplification of the experiment with hypertonic saline, and the fourth of the injection of isotonic glucose.

Osmolal concentration of body	= 330 mosM/kg. of water		
	Extra-cellular	Intra-cellular	Total
Water content of a 70 kg. body (liters)	= 12.5	28.5	41.0
Osmolal content of a 70 kg. body (mosM)	= 4,100	9,400	13,500

1. *Isotonic edema*: increase in intercellular fluid (caused by addition of  $\text{NaCl}$  in isosmolal solution).

11.7 gm.  $\text{NaCl}$  = 200 mM = 400 mosM.

$$\frac{400}{330} = 1.2 \text{ l. of isosmolal solution.}$$

Addition of this to the extracellular fluid increases it to 13.7 l., which is an increase of 10 per cent in the extracellular fluid, or of 3.4 per cent in total fluid.

2. *Isotonic dehydration*: decrease in intercellular fluid (caused by removal of NaCl in isosmolal solution). This is the reverse of example 1.
3. *Hypertonic edema*: increase of concentration of body fluids and shift of water from cells to intercellular fluid (caused by addition of NaCl without water). Addition of 400 mosM to the 4100 mosM of the extracellular fluid increases the content to 4500, and the concentration to  $\frac{4500}{12.5} = 360 \text{ mosM/kg.}$

But to equalize the pressure within and without the cells, water is transferred from the cells, thereby concentrating the solution within, and diluting the extracellular solution.

Therefore the concentration does not remain at the above figure, but must be calculated in terms of the whole body water:

$$400 \div 13,500 = 13,900 \text{ mosM in the whole body, and } \frac{13,900}{41} = 340 \text{ mosM/l.}$$

To reach this concentration the extracellular fluid is increased to  $\frac{4500}{340} = 13.2 \text{ l.}$

Thus by a transfer of 700 cc. of water from the cells osmotic equilibrium is reestablished at 340 mosM/l., the extracellular volume is increased 5 per cent, and the cellular water, because of its larger amount, is decreased only 2 per cent.

4. *Hypotonic dehydration*: decrease of concentration of body fluids and shift of water from intercellular fluid to cells (caused by removal of NaCl without water). Removal of NaCl without water must reduce the osmotic pressure:  $13,500 - 400 = 13,100; \frac{13,100}{41} = 320 \text{ mosM/kg.}$

If the total loss is in the extracellular fluid,  $4100 - 400 = 3700 \text{ mosM.}$

$$\frac{3700}{320} = 11.5 \text{ l. of extracellular fluid remaining.}$$

Thus 700 cc. of water is transferred to the cells, the concentration is reduced to 320 mosM/l., the cell water volume increased by 2 per cent and the extracellular water decreased by 5 per cent.

On the basis of these calculations a diagram has been constructed (Figure 9) slightly modified in principle from that of Darrow and Yan-net<sup>29</sup> and also extended to include examples 1 and 2. This diagrammatic representation is of course an oversimplification of physiological conditions, because in the body total fluid volume and concentration changes may occur simultaneously, and further because the unequal loss of Na<sup>+</sup> and Cl<sup>-</sup> may result in the additional factor of acidosis or alkalosis. There is at present no term to describe the condition of plethora of total base in the third example above (see *Total Base*, p. 120), and it is attained aside from experimental conditions only when high intake of salt is accompanied by limitation of water. In Darrow's experiment, following injection of hypertonic saline solution, no unusual clinical symp-

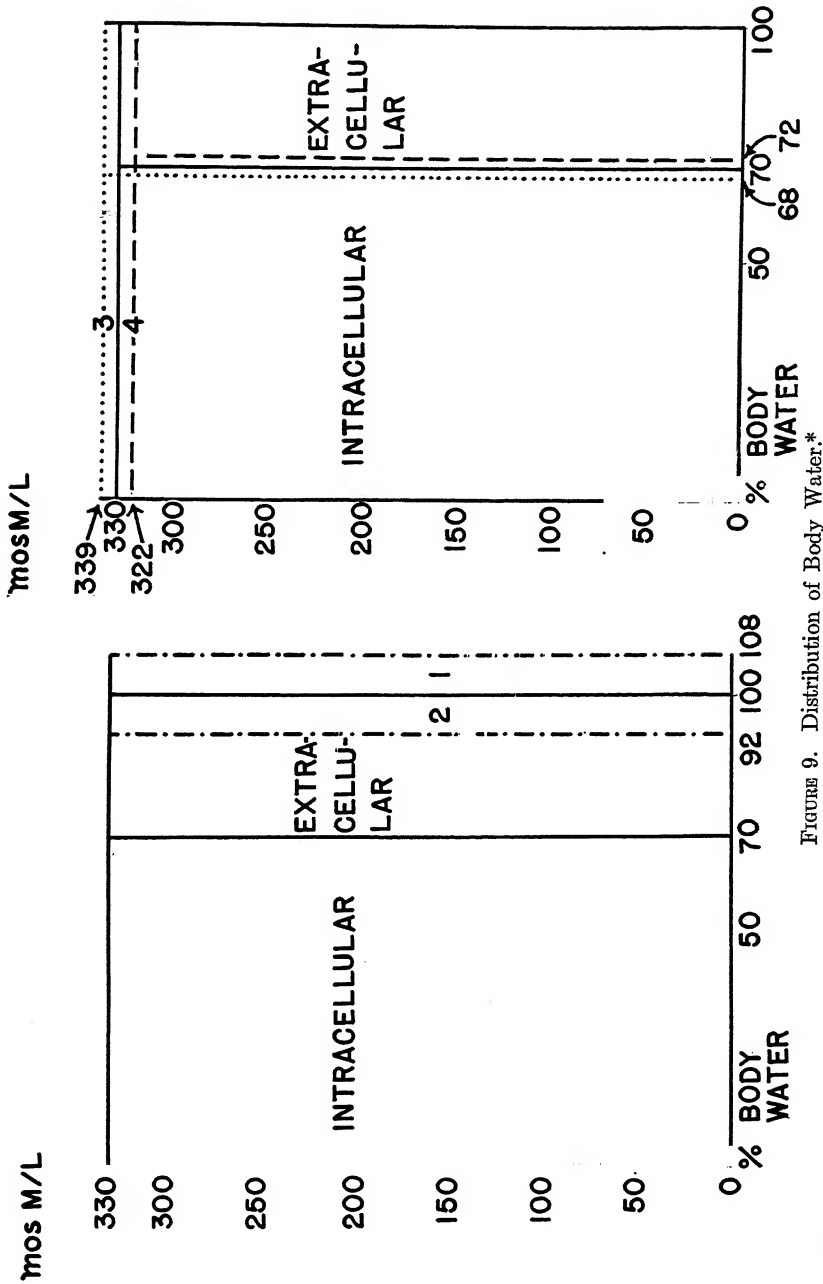


FIGURE 9. Distribution of Body Water.\*

\* From the examples given in the text, p. 98.

1. Isotonic edema: addition of 1.2 l. of isosmolar solution of NaCl.
2. Isotonic dehydration: removal of isosmolar solution of NaCl.

3. Hypertonic edema: addition of NaCl without water.
4. Hypotonic dehydration: removal of NaCl without water.

toms were observed. In two animals only was there a marked diuresis; in the rest there was anuria.

In the case of glucose injection, which caused decrease of intercellular fluid, anuria was observed. The symptoms closely resembled those of dehydration. But the loss of intercellular fluid was very small; the main alteration was its lowered concentration. Similar symptoms have been described earlier as resulting from loss of intestinal secretions, which causes dehydration. However these two types of dehydration must be clearly differentiated, for although there is a loss of  $\text{Na}^+$  and  $\text{Cl}^-$  from the interstitial fluid in both cases, hypotonic dehydration occurs chiefly by decrease in the  $[\text{Na}^+]$  and  $[\text{Cl}^-]$ , and isotonic dehydration by decrease in the amount of the intercellular fluid.

When a large amount of water is taken after dehydration, as in miner's cramps or, as in water intoxication, without salt, the osmotic pressure of the extracellular fluid must diminish and its volume increase, a condition not shown in the graph. This condition was observed clinically by Sunderman and Williams<sup>111</sup> in diabetics (but not in normal persons) when a large amount of hypertonic glucose solution (75 gm. in 200 cc.) was taken by mouth. The glucose could not be metabolized normally, but increased the osmotic pressure of the blood. This caused, 90 minutes later, a dilution of the intercellular fluid and a transfer of about a liter of hypotonic interstitial fluid to the blood.

The above discussion illustrates how the interstitial body fluid acts as intermediary between the cells and the secretions and excretions in relation to the material ingested. The exchanges through the medium of intercellular fluid are the mechanisms which correlate and integrate the various activities of the minerals in the body. The complicated relations between inter- and intracellular fluids are discussed also under *Fasting* (p. 308) and *Adrenal Glands* (p. 107).

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## Chapter 4

### Internal Secretions

The endocrine glands have no outlet to the exterior or to the intestinal tract. Their secretions consist primarily of organic substances which are absorbed into the blood. Because these secretions are transported to distant parts of the body where they exert physiological effects they have been called hormones. The isolation and determination of the chemical identity of some of them have been brilliant achievements in organic chemistry. For the purposes of this review they are of interest not primarily because of the minerals which they contain, but because of their effects upon water and mineral metabolism.

The modern developments in the physiology of internal secretions have demonstrated, as was long suspected, that each interacts with the others in a very complex manner. Thus the pituitary is related to the secretory activity of the thyroid, pancreas, sex glands and adrenals. A gland may produce several active principles. The effect of each secretion must be sought before and after removal of one or more of the other glands. The possible permutations and combinations should keep a whole generation of endocrinologists, physiologists and biochemists busy.

The following brief account of the internal secretions is mainly for orientation. Their chief function in relation to mineral metabolism is the regulation of concentrations of minerals in the blood, and of their excretion—especially sodium, potassium, calcium and chlorine. To this phase we have limited our discussion. Recent reviews have been given in the *Annual Review of Biochemistry*.<sup>15, 25, 31, 47</sup>

#### ADRENAL GLANDS

The adrenal glands are essential to life. Nearly a century ago Addison described a clinical syndrome due to pathological, often tuberculous, changes in this gland. This condition is closely simulated by experimental adrenal insufficiency in animals, except for the bronzing of the skin of human beings, due to deposits of melanin. Histologically the gland is differentiated into two parts, from each of which has been obtained an active principle.

**Medulla.**—Epinephrin, adrenin, or adrenalin was the first chemical hormone isolated and synthesized. Its chief actions are to accelerate the heart rate, to raise the blood pressure through its constricting action

on the arterioles, and to increase the blood sugar content. In the latter role it acts as an antagonist to insulin. As in other cases when carbohydrate is metabolized, the rise in blood sugar is accompanied by a fall in inorganic phosphate in the blood, and a rise of phosphate in muscles and liver. In diabetes, when sugar is not utilized, the fall in phosphate does not occur. When the blood sugar returns to normal the serum phosphate rises again and phosphate is increased in the urine. Simultaneously with fall in serum phosphate there is a transient rise in serum calcium. Adrenalin causes a lowering of bicarbonate in the blood, and an increase of lactic acid. It leads to formation of ketone bodies. After injection the effects are immediate but transient. Cannon<sup>12</sup> has described the effects on the body as those necessary for emergency action under stress or emotional states.

**Cortex.**—Adrenalectomy causes lowered blood pressure, hemoconcentration, loss of weight, diarrhea and great muscle weakness. After a period of anorexia and lethargy animals die within a period of several days to two weeks. This course of events is not favorably altered by administration of adrenalin. Physiologically active extracts of the adrenal cortex have recently been prepared. By their use adrenalectomized animals can be maintained in a normal condition. It is now possible by means of withdrawal and substitution to study the specific effects of deficiency and recovery uncomplicated by those of surgical procedure.

Chemical investigations of adrenal insufficiency show marked alterations in the mineral metabolism. In the blood the red cell count and serum protein are increased, which indicate a marked reduction of plasma volume. The non-protein nitrogen in the blood is increased as much as tenfold. The serum sodium is diminished to the extremely low value of 120 meq./l. (normally 145) and the potassium increased to 15 meq./l. (normally 4).<sup>22, 27</sup> The chloride and bicarbonate are reduced in proportion to the lowering of the total cation concentration. The increase in concentration of urea, sulfate, phosphate and potassium has been described as a compensatory mechanism to maintain osmotic pressure, rather than as a result of kidney insufficiency, a probably incorrect hypothesis.

The explanation of these phenomena has been found in the excretion of  $\text{Na}^+$  and  $\text{Cl}^-$  and associated body water in the urine, for in the absence of the hormone the normal conservation of these ions is lacking.<sup>21, 28</sup> The lowered value of  $[\text{Na}^+]$  and  $[\text{Cl}^-]$  in the blood reflects the condition throughout the intercellular fluids, and the state of dehydration and lowered osmotic pressure thus produced causes a shift in body water to the cells, thereby equalizing the pressure within and without. Decrease of extracellular fluid by intraperitoneal injection of glucose, although it has no symptomatic effect on normal animals, causes death of adrenalectomized animals.

Although the interrelations between  $[Na^+]$ ,  $[Cl^-]$  and body water are of great importance, the  $[K^+]$  changes probably are equally so. The amount of increase of potassium in the serum cannot in itself account for the symptoms of adrenal insufficiency, for similar increases of potassium under other conditions do not give rise to similar symptoms. The potassium in muscle is increased as well as that in blood—this in spite of the fact that there is a definite shift of water into the cells. The increase in cellular potassium under conditions of cell hydration indicates that in adrenal insufficiency some of the potassium must be non-ionized or in osmotically inactive form. Ingestion or injection of potassium intensifies the severity of the symptoms of insufficiency, and potassium-poor diets prolong life.

Remission can be brought about by the administration of NaCl intravenously or by mouth, so long as the intake is sufficient to overbalance the losses. When NaCl is given not only do the  $[Na^+]$  and  $[Cl^-]$  in the blood serum return to normal, but the serum  $[K^+]$  is lowered, and  $K^+$  is excreted in the urine. The relief due to the empirical use of NaCl injections by Rogoff and Stewart<sup>37</sup> is now understandable. There is thus disclosed a striking relation between sodium and potassium metabolism. Long ago Bunge stated that ingestion of potassium causes excess excretion of sodium, and *vice versa* ingestion of NaCl causes loss of potassium from the body. The validity of this finding is questionable in normals, but in cases of adrenal insufficiency is clearly demonstrable.

The administration of cortical extract produces startling recovery after a very short time. Animals, even in a fasting condition, recover within a few hours after injection. The effect of the hormone is augmented when NaCl is given simultaneously. The mechanism of the effect includes, then, both increase of the  $[Na^+]$  and  $[Cl^-]$  in intercellular water and decrease of intracellular potassium.

Harrison and Darrow feel that reduction of the extracellular water is not the deciding factor in the production of symptoms. In contradistinction to man, and also the dog and cat, the rat shows the complete chain of symptoms of adrenal insufficiency until death without marked changes in the distribution of the intra- and extracellular water.<sup>20</sup> Adrenalectomy may result in a rise of serum  $[Na^+]$  and  $[Cl^-]$  in the marmot and in the opossum.<sup>44</sup> The hormone affects the distribution of body water only through increasing the  $[Na^+]$  and  $[Cl^-]$  in the extracellular fluid, which in turn draws water from the cells, bringing the relation back to normal. Swingle *et al.*<sup>42</sup> showed that adrenalectomized dogs improved rapidly with cortical extract before any increase in the sodium of the serum was observed. On the other hand, withdrawal of cortical extract caused severe symptoms of adrenal insufficiency although, because of anuria, there was no significant reduction in the  $[Na^+]$  of the serum. In rapid recovery experiments, if the volume of the extracellular fluids increased

at the expense of the cells there would be a lowering of the  $[Na^+]$  and  $[Cl^-]$  in the serum. Whether the mobilization of potassium is effected directly by the action of the hormone or indirectly through the  $[Na^+]$  and  $[Cl^-]$  is not clear.

Recently Verzár (cited by <sup>31</sup>) has suggested a still more deep-seated function of the adrenal cortical hormone, namely the normal phosphorylation of fat and glucose. He claims further that it phosphorylates flavin (vitamin  $B_2$ ) and hence its absence results in a secondary avitaminosis. He states that adrenalectomized animals resemble those poisoned with iodoacetic acid. (See also Chapter 7, *Phosphorus*). The adrenal gland is the richest depot of cevitamic acid (vitamin C) in the body, but its relation to vitamin C metabolism is unknown.

One of the functions of the pituitary gland is its effect on the adrenals. Injections of anterior hypophysis have been shown to be of benefit both in animals without adrenals and in patients with Addison's disease. There is probably a stimulation of hypophysial function by the adrenals. Cortical hormone is related in its chemical properties to the male sex hormone. Tumors of the adrenals may result in hyperactivity and cause precocious sexual development. Whether this acts through the pituitary or not has not been ascertained. It has been suggested that the adrenals are of importance in the development of thyroid disease.

### PITUITARY GLAND

The pituitary gland, or hypophysis, consists of three parts: the anterior lobe, pars intermedia, and posterior lobe. At least eight principles are known to occur in the anterior lobe and two in the posterior. Various preparations involving partial separation have been produced for experimental studies. Knowledge about the secretions of the gland is at present in a ferment of development.

**Anterior lobe.**—Of the active substances secreted by the anterior lobe only the growth factor is mentioned here. In true dwarfs the gland is rudimentary. Hypoactivity leads to a condition called adiposogenital dystrophy. The relation of hyperactivity to abnormal bone development has long been known. The resulting conditions are gigantism and acromegaly. If the increase in hormonal activity precedes the closure of the epiphyses, as in the former, there is general overdevelopment of the bones; if it follows, the growth results in disproportion of the bones. Exostoses and tufts on the phalanges occur, resulting in enormous hands and feet. The lower jaw also increases to large size.

Our knowledge of the mechanism of the effects of this secretion on normal and pathological phases of bone development is meager. The activity is in some way related to blood calcium. In the hypoactivity associated with pseudoeunuchism the blood calcium is reported as

increased, and in acromegaly as lowered. Various conditions of the sex glands presumably acting through the pituitary are also reported in lower animals as affecting the blood calcium. However the literature is very controversial, and the effects in man are probably negligible.

**Posterior lobe.**—Pituitrin, the secretion of the posterior lobe, contains two active principles. The first, the oxytocic principle, causes marked contraction of smooth muscle, especially of the uterus. The other, called pitressin, causes contraction of other than smooth muscles, and acts as an antidiuretic.<sup>43, 49</sup>

The action of pitressin may be demonstrated on normal animals. Without it normal resorption of water in the kidneys does not take place, and its administration leads to reduction of the volume of urine. The effect is most obvious when extra fluid is given. Salt additions are without effect on such oliguria. The hormone acts directly on the kidney. Part of the action may be on the blood vessels, but not on the nervous control. Antidiuresis is evidenced even after the severing of all the kidney nerves, or when the isolated kidney is perfused.<sup>11</sup> This action of pituitrin has been attributed to stimulation of water resorption in the loop of Henle. The amount of antidiuresis caused in normals is quite variable. The response is much reduced during anesthesia or even during sleep.

**Diabetes insipidus.**—Patients with the rare clinical condition diabetes insipidus have lesions in or near the hypophysis. They excrete large volumes of dilute urine of low specific gravity and low mineral content. They are very thirsty and drink large volumes of water. Even when water intake is restricted the urine remains dilute, for they have lost the normal power to increase the  $[Na^+]$  and  $[Cl^-]$ . There are two types of diabetes insipidus.<sup>14</sup> In the first, water deprivation does not cause concentration of the urine, and dehydration of the body and increased blood chloride result. In the second type, partial power of concentration remains, and  $Na^+$  and  $Cl^-$  are excreted more readily than water. This results in a lowered blood chloride, which increases to normal or above in thirst. If  $NaCl$  intake is limited together with water intake, it is not necessary for them to form so much urine in order to keep the body in a normal state.<sup>48</sup> The hydration has not been shown to result in a material shift of water from the intercellular spaces to the cells. That the defect is mainly in the water metabolism is indicated by the fact that there are surprisingly small changes in the blood or in the amount of  $Na^+$  and  $Cl^-$  excreted in the urine. When posterior hormone is given, the patients show ability to excrete normal quantities and concentrations of urine. Ingestion of potassium increases the effect of posterior hormone and ingestion of calcium diminishes it.

In epilepsy when water accumulates in the body convulsions occur, especially when the excretion of potassium is greater than of sodium.



When such states were induced in epileptics by the administration of pitressin the loss of potassium was marked and the antidiuresis was followed by numerous convulsions.<sup>30</sup>

### PARATHYROID GLANDS

The removal of the parathyroid glands by Gley in 1892 and later by MacCallum and Vöegelin,<sup>29</sup> and the preparation by Collip<sup>13</sup> of the active principle, afforded means of showing by deficiency and excess the great importance of these small glands in calcium and phosphorus metabolism. The narrow limits within which the blood calcium (and to a less extent the phosphate) fluctuates with complete physiological disaster at either extreme illustrate the remarkable mechanism for the preservation of stability, and the importance of the small amounts of these minerals in blood serum. Because it is at present impossible to describe these changes adequately in chemical terms it is necessary to detail the physiological effects observed.<sup>40, 46</sup>

**Hypoparathyroidism.**—*Calcium and phosphorus.*—Removal of the parathyroids reduces the serum calcium to about one-half the normal amount. Tetany follows, and in many species death results. That the parathyroid hormone not only governs but is in turn governed by calcium and phosphorus metabolism is clear. Administration of calcium salts, whether by mouth or intravenously, abolishes the symptoms, raises serum calcium and increases phosphate excretion, chiefly by way of the bowel. Phosphates intensify the symptoms. Greenwald<sup>18</sup> observed that phosphate retention always followed parathyroidectomy. Shelling<sup>39</sup> was able to show that in parathyroidectomized rats the Ca/P ratio of the diet was the factor which determined the presence or absence of tetany. A low calcium-high phosphorus diet prevented it.<sup>35</sup> Salvesen<sup>38</sup> had previously shown in dogs, in the state of latent tetany caused by removal of most of the parathyroid tissue, that meat (high phosphorus) or milk with the calcium removed, caused tetany, but that whole milk prevented it. This experiment is not so clear-cut, for milk is high in both calcium and phosphate, and also in lactose. Other forms of tetany are also related to the Ca/P ratio (see *Tetany*, p. 159).

*Carbohydrate.*—Lactose alone will prevent tetany in animals without parathyroids.<sup>33</sup> The thesis that this neutralizes the effects of guanidine or other toxic substances is, in our opinion, not well founded. Because of the well-known relation of glucose to phosphorus metabolism, lactose probably acts in part by lowering serum phosphate. Also the fermentation of unabsorbed lactose produces acid substances in the intestine which, in turn, affect the absorption of calcium and phosphorus.

*Acid-base equilibrium.*—Hypoparathyroidism has an important effect on the acid-base equilibrium. Frank alkalosis is absent in this as in most

other forms of tetany. If present it intensifies the condition. Diminished excretion of  $\text{NH}_4^+$  in the urine and diminished gastric acidity are however, present. Ingestion of acids and of  $\text{NH}_4\text{Cl}$  or  $\text{CaCl}_2$ , which in the body act as acids, gives relief from tetany. Greenwald<sup>18</sup> suggested that such substances cause increased phosphate excretion in the urine. The implication is that, while not a primary cause of tetany, the acid-base equilibrium is one of the factors in the complex system. Both increased acidity of the blood and ingestion of acid tend to cause increase of  $[\text{Ca}^{++}]$ .

*Parathyroid hormone.*—With parathyroid extract Collip<sup>13</sup> was able to raise the serum calcium of parathyroidectomized animals to normal, to abolish symptoms of tetany and to restore normal health. But after long-continued administration parathyroid extract fails to relieve tetany.<sup>5</sup> The formation of antibodies has been suggested as an explanation of this.

*Vitamin D.*—That vitamin D will cure or prevent parathyroid tetany has been amply proved. Large doses are sometimes required. Taylor *et al.*<sup>45</sup> prevented tetany with vitamin D after the usual operation, but not after complete removal of glandular tissue. Vitamin D has been said to bring about the rise in serum calcium by increasing the "net absorption" of calcium, but it has been further shown that it acts when the intestines are removed or when the food contains no calcium.<sup>39</sup>

*A. T. 10 (Dihydrotachysterol).*—Holtz *et al.*<sup>23</sup> have shown that another irradiation product of ergosterol, A. T. 10 (anti-tetany), which does not cure rickets, is remarkably effective in raising serum calcium and abolishing tetany in hypoparathyroid conditions, both clinical and experimental. Like vitamin D, this agent does not lose its effectiveness with long use.

*Clinical.*—In addition to the experimental and surgical removal of parathyroids, a condition of hypoparathyroidism is seen as a rare clinical entity.<sup>8</sup> Conditions other than tetany which accompany this are brittleness of nails, thinning of hair, and cataract formation. Relief follows the measures found useful in similar experimental conditions, namely, parathyroid extract, high calcium diet, vitamin D and the new product, A. T. 10.

*Hyperparathyroidism.*—By injection of parathyroid extract Collip<sup>13</sup> produced hyperparathyroidism in normal dogs. In this condition the serum calcium is raised to 20 mg./100 cc. or higher. Symptoms of lethargy, muscular weakness, vomiting and diarrhea and thickening of the blood follow, and the serum phosphate, which is depressed at first, rises before death ensues. Following administration of the hormone, the course of events found by Albright *et al.*<sup>2</sup> was: first, an excretion of phosphate in the urine, and a lowering of serum phosphate; then a gradual rise in serum calcium and magnesium,<sup>17</sup> and later an increase in calcium excretion in the urine. The primary effect is thus found to be on the

phosphate metabolism. Negative balances of calcium ensue; calcium and phosphate are drawn from the bones. With excretion of minerals extra water is also excreted. Parathyroid extract has been used as a diuretic in cases of edema.

If administration of large amounts of the hormone is continued, bone rarification and osteitis fibrosa cystica occur.<sup>10</sup> The most rapidly growing bone is first drawn upon. This explains the maximal effects produced on both bone and serum calcium in young animals. In the rat, which is highly resistant to hyperparathyroid effect, huge doses produce osteitis fibrosa, but slightly smaller ones cause an increased growth of cartilage and osteoblastic activity, so that the bone trabeculae are increased.<sup>41</sup> The calcium withdrawn from the bones in hyperparathyroidosis causes not only increase in blood calcium and increased excretion, but also precipitation in the tissues, metastatic calcification (which see, p. 154).

Thus this condition constitutes almost the exact reverse of that seen in deficiency, not only symptomatically but also physiologically. High calcium intake intensifies, and high phosphate diminishes, the effects. Alkaline substances ameliorate the untoward effect.

*Clinical.*—Clinically hyperparathyroidism has only recently been recognized. A small but rapidly increasing number of cases have been reported. They show the symptoms already described. These have been found to be associated with parathyroid tumor and osteitis fibrosa. X-ray and surgery lead to cure. In the latter case the successful removal is often followed by tetany, presumably related to that described after withdrawal of active administration of the hormone.<sup>1, 7, 26</sup> This tetany occurs at a level of  $[Ca^{++}]$  in the serum which is above that usually associated with tetany. This has been interpreted as due to reversal of the previous flow from the bones to the blood, so that calcium is precipitated faster than it is introduced into the blood stream.<sup>9</sup> Aub has calculated that normally the body produces the equivalent of 50-75 international units of hormone per day, and that in hyperparathyroidism as much as 500 units are formed.

**Relation between parathyroid hormone and vitamin D.**—Many have raised the question whether vitamin D acts by stimulating parathyroid secretion or by enhancing its activity, or whether their actions are independent. Many experiments have shown that vitamin D draws calcium from the bones only when given in toxic doses; normally it increases absorption of calcium, but parathyroid always draws it from the bones. Vitamin D cures rickets, whereas parathyroid does not. Rickets can be produced and cured in animals without parathyroid glands. Increase of vitamin D intensifies the action of the hormone in raising blood calcium and causing abnormal calcification; without vitamin D this parathyroid action is lessened. Although parathyroid deficiency causes diminished serum calcium, absence of vitamin D does not. Both parathyroid and

vitamin D raise blood calcium, but the former lowers blood phosphate (except *in extremis*) whereas the latter raises it. Therefore, in spite of their common relation to calcium and phosphate metabolism, the action of the two is essentially dissimilar.

Further interrelation of vitamin D and parathyroid hormone seems probable. Under conditions such as the lack of vitamin D, when through faulty absorption of calcium, the blood level falls, a stimulation of parathyroid activity occurs, so that the normal calcium level in the serum is maintained. Hamilton and Schwartz<sup>19</sup> have demonstrated this condition in rickets (see *Rickets*).

In toxic doses parathyroid hormone, A. T. 10, and vitamin D all cause an increase in serum calcium, and all three lead to metastatic calcification. Recent studies by Albright *et al.*<sup>3, 4</sup> have shown how each of these separate agents in therapeutic amounts leads to a different quantitative response in calcium and phosphate metabolism, not only with regard to levels in the blood, but also in absorption of calcium from the intestine and excretion of phosphate in the urine. The following table from their data summarizes these different actions:

	Ca in serum	Phosphate in serum	Ca absorption	Phosphate excretion
Vitamin D	+	+++	+++	+
A. T. 10	++	++	+	+++
Parathyroid hormone	+++	-	0	++++

**Mechanism of parathyroid action.**—It has been found<sup>9, 24</sup> that, 48 hours after large doses of parathyroid extract with or without added phosphates, a hypocalcemia accompanied by tetany occurred. This has been interpreted as due to reprecipitation in the bones.

How these different effects are accomplished is unknown, chemically. Little knowledge has been gained by studies of dialysates and ultrafiltrates. All fractions of calcium diminish in parathyroid tetany, and all increase with excess hormone, or vitamin D, or calcium injection. For the present it is not profitable to speculate concerning possible ion and colloidal groups (see p. 134).

**Cerebrospinal fluid calcium.**—Cerebrospinal fluid calcium, which is normally one-half the value of the serum calcium, shows very small fluctuations whether in a condition of hypo- or hyperparathyroidism. Experiments in which the serum was dialyzed against cerebrospinal fluid indicated that parathyroid hormone had no effect upon the distribution of calcium between the two fluids.<sup>34, 36</sup>

#### THYROID GLAND

The active principle of the thyroid gland has been isolated by Kendall and Harington, and proved to be the iodine-containing peptid, thyroxin.

Iodine intake is a determining factor in the function of the thyroid gland. The relations between iodine metabolism and the thyroid are discussed in Chapter 10, *Iodine*.

The relation of the thyroid gland to basal metabolic rate is so striking that only recently has attention been drawn to its effect on calcium and phosphate metabolism. Aub and associates<sup>6</sup> were able to show that in hyperthyroidism the bones are rarefied; in cretinism or congenital hypothyroidism the bones show curious bands of density which have been attributed to cycles in thyroid activity. In the course of hyperthyroidism, or following thyroxin administration, large amounts of calcium, up to eight times the normal, are excreted, largely by the bowel. This is accomplished without any increase in blood calcium. How the fractions of calcium in the serum are altered or how this excretion takes place has not been explained. Only in parathyroid tetany does thyroid administration elevate serum calcium. Tetany is often produced by thyroparathyroidectomy, and therefore the separate effects of the thyroid and parathyroid have been confused.

The relation of thyroid activity to intercellular water is obvious from the name of a clinical form of hypothyroidism, myxedema. There is retention of fluid in patients with this type of thyroid deficiency. Administration of thyroid dissipates this edema and causes excretion of  $\text{Na}^+$ ,  $\text{Cl}^-$  and water in the urine.<sup>32</sup> In myxedema there is also retention of proteins whose end products are found in the urine following thyroid administration.

A recent study by Fellenberg and Grüter<sup>18</sup> has shown that, after removal of the thyroid glands of goats, the P/Ca ratio of the milk secreted is increased, the chloride is increased, and the ash is acid, and that these changes are reversed by administration of anterior pituitary hormone to the animals.

#### OTHER GLANDS

In this brief account we have made no mention of the internal secretion of the pancreas, insulin. Not only are the far-reaching effects of this hormone vital to the action of the other hormones, but its fundamental importance to the combustion of sugar in the body leaves no doubt as to its secondary effects on mineral metabolism, especially to phosphorus (which see). A proper discussion of the action of insulin is, however, too far afield for inclusion in this volume. Its description properly belongs to a treatise on diabetes mellitus.

A vast literature has accumulated concerning the internal secretions of the thymus and pineal glands, the ovaries and testicles. These are related not only to the actions of physiological systems, but also to many phases of mineral metabolism. Their connection with the development of secondary sex characteristics, and of cancer, indicate that their main

activity is biological and does not primarily concern mineral metabolism. All these secretions and also those of the spleen and lymph glands have been reported as affecting calcium and phosphorus metabolism.<sup>47, 50</sup> What little is known of these effects may be found in treatises on endocrine glands.

*Addendum.*—Recent experience with potent preparations of female and male sex hormones demonstrates their undoubted importance in relation to bone development, and hence to calcium and phosphorus metabolism. Further work reported at the Boston meetings of the American Society of Anatomists in March, 1939, by Gardner and Pfeiffer, confirms and extends their earlier work.<sup>16a</sup> Mice given estradiol show a great proliferation of osteoblasts, so that the bones appear very dense on x-ray examination and on histologic section. The effect of estradiol is counteracted by the male sex hormone. Administration of estradiol to chickens raises the [Ca] in serum above 75 mg./100 cc.

Dr. Fuller Albright\* has found that estradiol caused improvement in a case of osteoporosis which followed artificial menopause. The calcium and phosphorus balances became markedly positive and resulted in increased deposition in the bones.

Dr. Nathan Talbot\* has demonstrated that 12.5  $\mu$ g. of estradiol, administered daily for 8-10 days to new-born rats, caused an increase in both the size and the number of the centers of ossification in the wrists and ankles.

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## Chapter 5

### Total Base, Chloride, Ammonium and Bicarbonate

From certain aspects it is immaterial what particular ions make up the total mineral content of any given fluid. The osmotic pressure is equal to the sum of the partial pressures, and the acid-base equilibrium is the resultant of all the anions and cations present. But anatomically or physiologically the individual ions are not interchangeable. It was shown in Chapter 2 that the individual parts of the body are specifically constructed; in order to obtain coördinated growth it is necessary to have definite proportions of the building materials. Individual ions affect protoplasmic systems quite differently. These effects are due not only to the specific nature of the ions themselves and their concentrations, but also to the relative amounts of other ions affecting the same system. The sodium, potassium, calcium and magnesium ions in the body fluids must be balanced (*i.e.*, in certain proportion to one another) in order that normal irritability and contractility may be produced.

The body contains exquisitely fine mechanisms for the maintenance of physiological constants, discussed in Chapters 3, 12 and 13. However, if the intake is abnormal either through excess or scarcity, absolutely or relatively, alterations in body composition must ensue; or if, through pathological conditions, the mechanisms of regulation are hindered, the relative concentrations in the body are changed. Thus in a fundamental manner the relationships of the various ions determine physiological activity. The following may be cited as examples of cases in which relative amounts of minerals involve special problems: Ca/P in bone formation; Na/K in distribution of body water; Ca/Mg, K/Ca, or Na + K/Ca + Mg in relation to irritability; K/P in muscle contraction; N/S and N/P in protein composition.

Before proceeding further with a discussion of physiological mechanisms it is well to summarize the activities of the individual elements. In other chapters they have been discussed as groups under given functions. It is also worth while to see the variety of functions of each from the point of view of the minerals rather than of the body systems. The material distributed throughout the book under other headings is thus recorrelated, but not elaborated here. In this chapter are considered the total base, the alkaline minerals sodium and potassium in relation to each other and to chloride, and ammonium and bicarbonate. This material has been reviewed.<sup>28, 32, 37, 43</sup>

## TOTAL BASE

The grouping together of all the electropositive minerals in the body (exclusive of  $\text{NH}_4^+$  and organic cations) has been a happy one. Not only can they be measured by a single determination, but this datum has useful applications. It has been customary to designate this analytical value as *total base*.

The total osmotic concentration of blood plasma is about 320 mosM/l. as determined by the freezing point.<sup>21, 22</sup> The total base by analysis is 155 meq./l., and the sum of the anions is 150 meq./l. The sum of the cations and the anions thus falls short of the total by little. The sugar, urea and other non-electrolytes may at times reach a much larger value than is represented by the difference. The specific differences of the various body fluids in regard to total base, both quantitative and qualitative, have been discussed under their respective headings. Part of the anions come from organic compounds and proteins, which may be only slightly ionized; and therefore systems having the same osmotic pressure but different protein contents contain different amounts of total base. In such cases, as for example the blood cells and serum, divergences from the normal osmotic pressure of both may be calculated from the analysis of the total base of either. This is discussed on pages 25 and 97.

The body fluids remain in osmotic and acid-base equilibrium with one another. Because the electrolytes exert their activity only in water solutions, better insight is obtained by measuring them in relation to the water content of the fluids. In spite of the proteinate concentration, the body fluids highest in protein contain the fewest cations per unit of volume (see Table 6, p. 22). This apparent paradox is made clear when the cations are computed per unit of water. On this basis the fluids highest in protein contain the most cations. The proteins of the red cells, for instance, account for about 30 per cent of the volume of the red cells so that the cations per unit volume are 23 per cent less than those of the cerebrospinal fluid, but per unit of water are 14 per cent more.

The constancy of the total base in any of the fluids, of which the serum is most accessible, gives an excellent insight into the tenacity with which the body maintains its osmotic pressure. The individual anions of the blood plasma are subject to large variations. The organic anions may increase as in diabetes, and cause a reduction of  $[\text{HCO}_3^-]$  and  $[\text{Cl}^-]$ ; hyperventilation may reduce the  $[\text{HCO}_3^-]$  and increase the  $[\text{Cl}^-]$ , or the reverse condition may be present; vomiting may deplete the plasma  $[\text{Cl}^-]$  to half. But it has been stated<sup>37b</sup> that the entire course of the anion relationships may be run with little or no change in the sum of the cations, or even in the individual cations.

If cations are lost from the body and water is also reduced, a minimum alteration in [cation] occurs. Similarly the volume of the inter-

stitial fluid increases when electrolytes are retained. To describe this phenomenon and also the exchange of water between cells and extracellular fluid Peters and Van Slyke have used the term "volume buffer" to indicate that the concentration is more fundamental than the volume of the body fluids. Gamble<sup>15a, 16</sup> has shown that in dehydration a loss occurs in both extracellular and intracellular water. By this means the body provides both minerals and water to meet its urgent needs without materially altering the concentration of electrolytes in the blood plasma, until *extremis*. The most frequent alteration in total base found clinically is the loss of mineral cations in dehydration, especially in those cases of diabetes and nephritis complicated by acidosis. Dehydration results from the loss of water and electrolytes from the body from any source. Even prolonged diuresis may cause a reduction of positive minerals. When vomiting is prolonged, as in obstruction of the pylorus or upper intestinal tract, because of the great loss of  $\text{Cl}^-$  and relative superabundance of cations, cations also are lost by excretion.<sup>17</sup>

More positive than negative mineral equivalents are normally lost by way of the intestinal tract. In the case of severe diarrhea or of intestinal fistula the loss of minerals may become excessive. If the losses of body fluids are great, there comes a time when the volume of the circulating plasma is diminished. Because the protein is not lost the concentration of plasma solids is increased. This increase compensates slightly for the loss of total base in the maintenance of osmotic pressure. Measurement of plasma protein is a better indication of plasma volume than of the loss of electrolytes, for the plasma volume is maintained by drawing the other body fluids into the blood stream. The loss of total base is best measured by balance experiments or by weight loss or other methods of measuring body water.

The extreme variations in concentration of total base of the serum, observed clinically, range from 120-180 meq./l. The lowest values of total base in the serum occur when water is administered without salt following previous depletion, for example, after dehydration or copious sweating. A similar condition, presumably due to dilution of the electrolytes in plasma, results from forced ingestion of an excessive amount of water, a condition called "water intoxication." To differentiate these conditions we have called them "hypotonic dehydration" or "hypotonic edema" (p. 98) depending on the volume of extracellular fluid. Increase of cations does occur, but the condition is extremely rare clinically. It results when salts are supplied and water restricted, or when alkalis are administered to patients with limited capacity to excrete them,<sup>30</sup> or when water is withheld in the treatment of diabetes insipidus. The highest concentration of total base in the plasma of which we are aware, 236 meq./l., resulted from feeding puppies concentrated milk.<sup>11</sup>

The role of the positive elements in body structure is discussed in

Chapter 2, in body fluids in Chapter 3, their relation to water retention and excretion in Chapters 3, 12 and 13, and their intake in relation to body functions in Chapter 13.

### SODIUM

Sodium is not only the largest fraction of the total base of the body fluids, but the  $\text{Na}^+$  together with the  $\text{Cl}^-$  are the main components which determine the osmotic equilibrium. As it occurs in the body, sodium is largely associated with  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , and even that fraction which occurs as proteinate is almost completely ionized. Inasmuch as it occurs in no special organic combinations whose metabolism is different from that of the body fluids, a separate discussion of its occurrence and function need not be given here.

Because sodium plays such an important part in the concentration of the body fluids, it is not surprising that experiments<sup>39</sup> with rats have shown that life cannot be supported or normal growth take place when the sodium intake is too low. The vegetable foods and muscle cuts of meat are all comparatively low in sodium and high in potassium. However, no human dietaries, even without added salt, are so low in sodium that they cannot support life. All human beings and herbivora gladly accept sodium as  $\text{NaCl}$  when it is attainable. The normal amounts consumed in this country seem to be of the order of 4 gm. of sodium, or 10 gm. of  $\text{NaCl}$  per day, and in Europe are apparently nearly twice this value. The maximum amount of  $\text{NaCl}$  that the average normal can take, without accumulating edema fluid, is about 35-40 gm. per day.

The sodium content of the adult is 60-65 gm. It occurs principally in the plasma and interstitial fluids. The amount within the red blood cells is so small that its presence has been questioned. This is not true of the blood cells of other species, in which sodium may be present in considerable amounts.<sup>27</sup> The calculations of Harrison, Darrow and Yan-net<sup>20</sup> have shown that some sodium must occur distributed throughout the cellular water, but this varies from species to species, and has not been ascertained for man. The  $[\text{Na}^+]$  in the muscle cells of dogs is not more than 10 meq./kg. of intracellular muscle water. Most of the sodium unaccounted for by interstitial fluids is in the skeleton, and cartilage has an especially high sodium content.

### POTASSIUM

Like sodium, potassium is essential to life. It is widely distributed in both plants and animals. The potassium content of the diet is of no practical concern for, in diets adequate in other respects, the potassium content is more than ample to meet the body's needs.<sup>33</sup> The average intake is 2-3 gm. per day.

Potassium occurs in the body in conjunction with phosphate, chloride and bicarbonate. Its distribution as the principal cation within the cells has been discussed in Chapter 2. The potassium content of the red corpuscles is about 170 meq./l. of water, and of the blood serum only 5 meq., a ratio of about 34/1. The chemical forces which restrain this within the cell are as yet unknown. Potassium added to the plasma does not diffuse into the cell across the cell membrane, if there is such a membrane. However there must be some mechanism as yet undisclosed to account for the entrance of potassium into and its egress from the cell. The intact muscle acts differently from that which has been separated from the body. The former seems to be impermeable to the mineral cation, and to phosphate and chloride, but isolated muscle is permeated by them.

Cellular potassium is associated with cellular phosphorus. Undoubtedly some of the  $K^+$  balances some of the complex phosphate anions. Some potassium has been reported as "bound." There seems little question that some of the potassium must be in a combination in which it is not freely ionized, and hence does not exert osmotic pressure apart from the whole molecule. Because of its high concentration in red cells, osmotic equilibrium between the cells and surrounding fluid would not be attained if all the potassium were ionized. Increase in interstitial water, as in ordinary edema, does not alter the cellular potassium or water content. During cellular activity the potassium is said to accumulate near the surface of the cells. The cell loses potassium during muscular activity and potassium feeding restores it.<sup>12, 13</sup>

When positive elements are lost from the body as a result of ingestion of acid or in fasting, the amount of potassium found in the urine is of the same order of magnitude as that of the sodium (see *Mineral Cationogen-Excess Balance and Fasting*). In fasting it is possible to attribute some of the potassium excreted to the protein loss resulting from cell destruction, but this does not account for all of it. It is necessary both in this case and in the case of acidosis to draw the conclusion that some of the potassium excreted is made available through the loss of intracellular body fluid (as well as extracellular). In recovery periods this lost potassium is rapidly restored.

The  $[K^+]$  of the serum is remarkably constant; ingestion of large amounts by mouth does not raise it. When it is increased by injection, increased amounts are found in the gastro-intestinal secretions (see p. 59). When injected intravenously in large amounts it may cause death.<sup>5, 6, 48</sup>

Ingested potassium bicarbonate and potassium salts of organic acids not only act as alkalis in the body, but show the characteristic  $K^+$  effect. The pharmacological action of potassium is to increase neuromuscular irritability. Such a condition resulted in cattle when the ratio of Na/K

ingested was 1/542. However, in the main clinical condition in which  $[K^+]$  is increased in the blood serum, namely Addison's disease, the picture is exactly the reverse, the symptoms being great lassitude and weakness. Therefore the increased  $[K^+]$  in the serum is only part of the clinical picture. Harrison and Darrow<sup>19</sup> have stated recently that, following removal of the adrenal glands, potassium accumulates within muscle cells, but not in those of the liver or brain. This is all the more striking because of the dilution of the other constituents within the cell. It seems too early to pass judgment on the relation of the potassium content of these cells to the weakness and fatigue in such animals.<sup>9, 10</sup>

A rare clinical condition has recently come to light, called "family paralysis."<sup>14, 38</sup> In this condition the  $[K^+]$  of the serum is extremely low and potassium administration by mouth causes alleviation of symptoms.

Ingestion of excess potassium is not as well borne as that of sodium. Blum *et al.*<sup>5</sup> state that not more than 25 gm. of KCl can be consumed without untoward symptoms. As was stated in Chapter 3, potassium salts are used as diuretics because the body has not the capacity for storing potassium in the interstitial fluid. But Peters and Van Slyke state that they have seen edema following ingestion of less than 20 gm. of KCl. Potassium salts are tolerated poorly by patients with cardiac decompensation and nephrosis. When difficulty of excretion of  $Na^+$  and  $Cl^-$  is present,  $K^+$  is also poorly excreted. Collapse has been reported in such patients after very small amounts of KCl.<sup>45</sup>

### SODIUM AND POTASSIUM

Bunge<sup>8</sup> made the observation that NaCl is universally desired when vegetable intakes (high potassium) are large. Hunters as well as animal husbandrymen know that herbivora come to salt licks. Bunge regarded such salt as a necessity, and not a condiment. He made an amusing calculation of the sodium and potassium intakes of a cat which ate a mouse and of an herbivorous animal, and came to the conclusion that the Na/K ratio in intake was the determining factor of the NaCl requirement. His conclusions, that excess of either potassium or sodium cause excretion of the other, were based upon short metabolism studies with sodium and potassium bicarbonates and citrates.<sup>7</sup> MacKay and Butler<sup>30</sup> have reviewed the existing evidence and from their own experiments showed that in moderate amounts either could be ingested without definite effect upon the retention of the other except perhaps for the first day. (See also Table 11, p. 80, and <sup>47</sup>.) Gamble<sup>15b</sup> found, in urine of maximum concentration, that the sodium and potassium were additive, so that the urine had the same concentration of cations, whether the mineral ingested was sodium, potassium, or any mixture of the two.

The body always contains more potassium than sodium, the difference increasing with growth. Therefore when growth is rapid an excess of potassium over sodium intake seems desirable. Milk provides such a ratio;  $\text{Na/K} = 1/2.5$  by weight, or  $1/1.5$  by equivalents. For adults the potassium requirement is at a minimum, while the desirability of higher  $\text{NaCl}$  consumption continues throughout life. The ratio of average intake of the adult is  $\text{Na/K} = 1.5/1$  by weight, or  $3/1$  by equivalents.

Herbivora which consume grass fodder have a dietary  $\text{Na/K}$  ratio of  $1/18$ , and the diet is alkaline. Unless this is supplemented with acid grains and  $\text{NaCl}$ , it is low in sodium both absolutely and relatively.<sup>41</sup> In this addition the chloride is as important as the sodium.

In the light of present knowledge Bunge's thesis may be stated in different terms. The carnivora have a high potassium diet. The sodium, derived mainly from the body fluids of the animals which they consume, is adequate and in the same proportion to potassium as in milk. Because of great muscular activity the excess of potassium meets their requirements. The herbivora on the other hand, have a low  $\text{NaCl}$  intake, but have a greater need for it because of their great digestive activity, and hence the avidity for salt additions. The liberal use of  $\text{NaCl}$  facilitates the formation of digestive juices, whose minerals must otherwise be withdrawn from other body fluids. Salt fulfills the same function in man. It permits a freer flow of saliva and gastric juice and an increased sense of well being. Therefore the universal use of  $\text{NaCl}$  in cooking and as a condiment seems to provide for a physiological function.

Because of the specific chemical composition of the various body fluids, losses or gains to the body stores can be adequately assessed in terms of extracellular and intracellular fluids on the basis of the sodium and potassium excreted.<sup>18</sup> The fundamental relationship is that, expressed in meq./kg. of water,  $[\text{Na}^+] + [\text{K}^+]$  represents the total amount of fluid, and  $\text{Na/K}$  the relation of extra- to intracellular fluid. See further under *Fasting* (p. 308). The original studies were made upon the urine of fasting subjects, but these values can be used in balance studies as an approximation of the distribution of body fluid either gained or lost. Sweat must be either maintained at a minimum or else accurately analyzed.

The following equations for the calculation, in balance studies, of the source of the urinary sodium and potassium, whether from muscles or extramuscle fluid, are modified from those of Peters and Van Slyke.<sup>37b</sup>

$[\text{Na}^+] \text{ in muscle fluid} = 0.425 [\text{K}^+] \text{ (expressed as meq.)}$

$[\text{K}^+] \text{ in extramuscle fluid} = 0.017 [\text{Na}^+]$

$[\text{Na}^+] \text{ of extracellular fluid} = 148 \text{ meq./l. of water}$

$[\text{K}^+] \text{ of muscle fluid} = 112 \text{ meq./l. of water}$

$$\frac{[\text{Na}^+] - 0.425 [\text{K}^+]}{148} = \text{liters of extramuscle fluid}$$

$$\frac{[\text{K}^+] - 0.017 [\text{Na}^+]}{112} = \text{liters of muscle fluid.}$$

With regard to the muscles themselves, Hastings and Eichelberger,<sup>24</sup> by analyses of the muscles of dogs, have computed that, of the muscle water, 17 per cent is extracellular, and 83 per cent within the muscle cells. They were able to show that with isotonic saline injections the interstitial fluid increased without change in the cellular fluid. The intracellular fluid was increased to a slight extent by injections of either alkaline or acid isotonic solutions of electrolytes, but the extracellular fluid was increased to a much greater extent by the alkaline than by the acid solutions.

Adrenal insufficiency is the only physiological condition in which the Na/K ratio may be shown to be of paramount importance. Here the close correlation between symptoms and Na/K of intake is striking. Either administration of large amounts of potassium or failure to give adequate sodium causes the latent condition to become manifest, or if the symptoms are already present, they are intensified and death may ensue. (See p. 107).

### CHLORIDE

Chlorine comprises about 0.12 per cent of the body of the adult. Throughout the body it is so intimately associated with sodium that it is often difficult to separate the two, and it is profitable to consider them simultaneously. Chloride occurs especially in the extracellular fluids. It is therefore to be found principally in tissues with most interstitial water, the skin, subcutaneous tissue, blood, muscles and bones. The  $[\text{Cl}^-]$  is greatest in those fluids which contain least protein. The cerebrospinal fluid contains the most chloride, then the interstitial fluids, the lymph, serous transudates, exudates and the gastrointestinal secretions. The blood plasma acts as the central depot for the formation of these fluids and for their resorption. It is also the source of the chlorides found in the urine, feces, sweat and milk. The plasma chloride is thus in equilibrium with all the body fluids, and there is a large transfer of chloride to and from the blood in the daily metabolic cycle.

$\text{Na}^+$  and  $\text{Cl}^-$  are the largest factors in the maintenance of the osmotic pressure of the extracellular fluids. Alterations in them cause changes in either volume or concentration of these fluids. The cellular water is in osmotic equilibrium with the extracellular water. Therefore the osmotic pressure is determined by and reflected in changes in the extracellular fluid. The relation of edema and dehydration to the amounts and concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  is discussed in Chapter 3 and in Chap-



ter 12, *Water Metabolism*. The action of acids in decreasing and of alkalis in increasing the volume of body fluids is described in Chapter 13.

Obviously when anions or cations are to be excreted the two ions of the ingested NaCl may act quite separately. But in edema fluid they are stored together, and the dissipation of the edema leads to their simultaneous excretion. That  $\text{Na}^+$  and  $\text{Cl}^-$  excretion also parallels the water balance is usually true, but it has been shown that in the course of pneumonia,<sup>2</sup> in a febrile cold<sup>1, 30</sup> or in infancy, they may be stored without edema, or with variable amounts of fluid. Under these conditions the  $\text{K}^+$  in the urine becomes a much larger percentage of the mineral cation excretion. The sudden excretion of the stored  $\text{Na}^+$  and  $\text{Cl}^-$  following the crisis in pneumonia has long been referred to as the "chloride crisis."

Although  $\text{Cl}^-$  occurs in all body secretions and excretions, in the gastric juice it is unique because the principal cation accompanying it is not one of the minerals, but  $\text{H}^+$ . The stomach has the capacity to secrete  $\text{H}^+$  and  $\text{Cl}^-$  in the same concentration as the chloride of the blood plasma (see *Gastric Juice*, p. 65). In the other alimentary secretions the  $\text{Cl}^-$  is the main anion. It is in a medium of  $\text{Na}^+$  and  $\text{Cl}^-$  that the enzymatic actions of digestion take place. So, too, these ions are the principal determinants of the osmotic pressure of these juices, and because of this they assist in the transfer of materials to and from the intestinal tract. Alterations in the blood  $\text{Cl}^-$  are reflected in these fluids. This subject has been discussed in Chapter 3 (p. 60).

It has gradually come to be known that chloride plays a negligible part within the tissue cells. It is questionable whether any chloride at all is found within the muscle cell. The relation of chloride to red blood cells is a special case. These cells are unique in regard to their high chloride content, which is about 55 per cent of that of the plasma, per unit volume. The capacity of the red cells to accept chloride from the plasma in exchange for bicarbonate has been called the "chloride shift."

The relation of acid-base alterations to oxidation and reduction of the blood cannot adequately be described without including the part played by chloride. As the blood becomes more alkaline or becomes oxygenated, the ratio of the chloride in the cells to that in the serum, calculated per unit of water, falls. The differences between the chloride distribution ratios in oxygenated and reduced blood are much less marked than those of bicarbonate. (See Table 25, p. 282.)

The plasma  $[\text{Cl}^-]$  is about 102 meq./l. and the  $[\text{HCO}_3^-]$  about 27 meq./l. Together they constitute the largest fraction of the anions, the rest being organic anions and proteinates. The sum of the two is more constant than either alone. In general the  $[\text{Cl}^-]$  tends to increase when the  $[\text{HCO}_3^-]$  is diminished, and *vice versa*.

The requirements for intake of sodium and chlorine cannot be defined separately. They are usually consumed as salt and excreted together.

The NaCl content of human dietaries, even without added salt, is certainly above the minimum requirement. On very low chloride intake growth is retarded or lacking, and animals are stunted. In most experiments of this sort however, other minerals were also limited. Large NaCl intakes lead to some retention and edema even in the normal adult. Very high salt intakes may be definitely harmful. Perhaps this comes from direct injury to the epithelial cells. Large intakes of NaCl lead to enlargement of the kidneys of experimental animals.

Ingested chloride is rapidly absorbed from the intestine and deposited temporarily in the skin and interstitial spaces. It is then excreted in the course of four or five hours. Chloride excretion has a normal diurnal variation; most is excreted by day. When the subject sleeps by day the findings are reversed.<sup>35, 44</sup> When the intake is diminished the urine becomes practically chloride-free. When intake is liberal the kidney eliminates the excess. Although the urine is normally the principal means for the removal of  $\text{Na}^+$  and  $\text{Cl}^-$ , they are also excreted in the feces and sweat. Under special circumstances any one of the three may become the dominant path of excretion. Whenever the excretion of  $\text{Na}^+$  and  $\text{Cl}^-$  in the sweat or feces is large, that in the urine is decreased.

The relation between salt intake and water intake is very important in the metabolism of sodium and chloride. With low salt-high protein intakes the volume of urine is governed by the urea excretion, and the usual amount of water is required for urine formation. With low salt-high water intakes minerals are swept out and water intoxication results (lowered osmotic pressure). With high salt-low water intakes the osmotic pressure of the body fluids is increased. This concentration leads to a condition called "salt fever." As little as 3 gm. of NaCl per day may produce this condition in infants if water intake is limited.

It is certain that in kidney disease of the nephrotic type the ability to excrete  $\text{Cl}^-$  is greatly diminished. Large or even normal amounts of NaCl in the diet result in the formation of edema, or in the increase in edema if already present. Low salt diets are used therapeutically to alleviate this condition. The primary reason for this type of edema is lowered serum protein. The oncotic pressure in the capillaries becomes inadequate to cause the return of the transudates to the blood vessels. The same effect can be produced by low protein diets or by plasmaphoresis.

On the theory that increased water in the tissues renders them less able to withstand infections, low NaCl diets have been recommended, especially in Germany, for treatment of skin tuberculosis and other skin infections.

Balance experiments with NaCl are exceedingly difficult. Although actual equilibrium may be attained at various levels, the sum of the  $\text{Na}^+$  and  $\text{Cl}^-$  found in urine and feces usually does not exceed 93-94 per

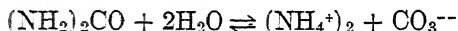
cent of the intake. Presumably this loss occurs in the sweat. Further, it may take two weeks for equilibrium to be established, even on constant diets and constant water intakes. The tides of adjustment seem to operate very slowly and with large, unaccountable fluctuations.

### AMMONIUM

Ammonium and bicarbonate, unlike the minerals, are formed in the body when, and to the extent, required by metabolic processes.

Ammonium is present in large amount in the urine. This must be elaborated by the kidney, because it is present only in traces in any of the organs or body fluids. The blood contains, at most, only 0.01-0.02 per cent. This does not vary, except perhaps *in extremis* of acidosis when the  $[H^+]$  is also changed. Nash and Benedict<sup>3, 34</sup> showed that the  $[NH_4^+]$  in the renal vein was higher than in the renal artery. The amount of  $NH_4^+$  in the urine has been calculated to be greater than the total amount contained in all the blood flowing through the kidneys.

The urea and nucleotide fractions of the plasma have been suggested as the source of  $NH_4^+$  in the urine. This is probably erroneous, for the kidney has not been shown to contain a urease which can convert urea into  $NH_4^+$  according to the equation:



Presumably ureases do occur in other parts of the body, for the ammoniacal breath of uremics has been attributed to such splitting of urea in the mouth. In infections of the bladder, bacteria rapidly convert urea into  $NH_4^+$ , as they do outside the body. The reverse mechanism takes place when ingested ammonium is converted to urea in the liver. Such a reaction may perhaps take place in other tissues also. The suggestion of Bliss<sup>4</sup> that ammonium may be derived from the amides has not received general confirmation. Parnas<sup>36</sup> and his associates have stated that there is an ammoniogenic substance in the blood, to the extent of about 2 mg. of N/100 cc. of plasma, which may be the source of the urinary  $NH_4^+$ . It is presumed that ammonium formation during muscle activity *in vivo* or in isolated muscles is bound to such a complex. It has recently been shown by Krebs<sup>29</sup> that the kidney contains deaminizing enzymes. This may be the principal mechanism for the formation of  $NH_4^+$  in the kidney.

Ammonium functions to facilitate the excretion of anions by the kidney with a minimal excretion of mineral cations. Walter showed in 1877<sup>46</sup> that ammonium excretion in the dog was greatly increased after acid poisoning. Salkowski<sup>40</sup> showed that acid-poisoned rabbits did not neutralize the acid with  $NH_4^+$  but with  $Na^+$  and  $K^+$ . This controversy

led to over a hundred experimental studies, the outcome of which proved that both were right. Carnivora possess a power to form ammonium, which herbivora lack. It finally came to be realized that the omnivorous human being follows a metabolic pattern between the two; part of the anions are neutralized by  $\text{NH}_4^+$  and part by mineral cations, and when there is excess acid to be excreted the two categories increase about equally.

The amount of ammonium in the urine closely parallels the amount of acid claiming excretion. Janney,<sup>26</sup> by suitable addition of alkali, reduced the urinary  $\text{NH}_4^+$  to the vanishing point. During the "alkaline tide" following gastric secretion, ammonium formation is depressed. The  $\text{NH}_4^+$  content of the urine is normally 20-30 meq./day. Of the anions not balanced by mineral cations roughly one-half are neutralized by  $\text{NH}_4^+$ , and one-half are excreted as "free acid."

Henderson and Palmer<sup>25</sup> showed that in some pathological conditions, especially in kidney disease, the capacity to form  $\text{NH}_4^+$  is limited and hence the body fails to eliminate the anions which require excretion. In diabetic acidosis and in some infections, where a large amount of organic acid is formed as a fault in metabolism, huge amounts of  $\text{NH}_4^+$  may be produced, up to 600 meq./day.

Considerable interest has been attached to the measurement of the fraction of nitrogen excreted in the urine as  $\text{NH}_4^+$ . Ordinarily this represents about 5 per cent of the total nitrogen. With large protein intakes, because they produce acids when metabolized, this proportion may be increased, for as shown above, the amount of  $\text{NH}_4^+$  depends primarily upon the anion excretion. Hasselbalch<sup>23</sup> has used this ratio as an index of acid excretion. When it was calculated under conditions of pH 5.8, he felt it was an index of the organism's ability to protect itself against acidosis; see Klink.<sup>28</sup>

The sum of the titratable acid and ammonium, which has been called the "total acid excretion," really represents that fraction of the anions above that which has been excreted neutralized by mineral cations; and the ratio of this total acid excretion to the mineral cation excretion represents the effectiveness of the body in conserving its positive minerals when acid is to be excreted.

During fasting the protein and fat metabolism result in sufficient acid end products to produce acidosis. Under these conditions Gamble was able to evaluate the role of  $\text{NH}_4^+$  and other factors. He found that the ammonium mechanism for sparing mineral cations is one that develops slowly, over a period of days. Similar results obtain when acid or acid-producing salts are fed.  $\text{NH}_4\text{Cl}$  is itself such an acid-producing salt. The  $\text{NH}_4^+$  is converted to urea and the  $\text{H}^+$  and  $\text{Cl}^-$  released must be excreted by the kidneys. This acid in turn calls for the production of

$\text{NH}_4^+$  to neutralize it in the urine. High ammonium excretion continues after the fast, or acid administration, is discontinued. This can be interpreted only as a further mechanism for the conservation of positive minerals.

Similar acid-producing salts are the sulfate and chloride of calcium and magnesium, for the alkaline earths are excreted mainly by the bowel, and the anions remain for excretion by the kidney, with consequent increase in ammonium formation. Phosphate causes less formation of ammonium, for here the excess anions are excreted principally as "free" or titratable acid. For further discussion of the mechanism of acid excretion (see p. 286).

#### BICARBONATE

Carbonic acid is formed when carbon is burned in the body and this acid is available in almost unlimited amounts. As it is formed in the body, it is not usually included among the anions when one considers them in terms of amounts ingested and excreted and the balance which results.

The amount of expired  $\text{CO}_2$  is truly amazing. It is simple to calculate, from the respiratory metabolism, that a man produces 12-15 liters of  $\text{CO}_2$  per hour, or 300-350 l./day. This is equivalent to 13-15 mols. If this were combined with base it would neutralize 300-350 gm. of sodium, to form  $\text{Na}^+$  and  $\text{HCO}_3^-$ . If all this acid had to be neutralized in the body the description of the mineral metabolism would be quite different.

The  $\text{CO}_2$  in the body fluids is present mainly as  $\text{HCO}_3^-$ . It is of prime importance in regard to maintenance of acid-base equilibrium of the blood and body fluids. Its reactions are so intricate that Peters and Van Slyke have devoted to it one-third of the space allotted to minerals. A brief discussion is given in Chapter 13, under *Acid-base Equilibrium*.

The importance of  $\text{HCO}_3^-$  in urinary excretion was clearly defined by Gamble. Because, like other anions, it can neutralize cations it is not surprising to find that the  $[\text{HCO}_3^-]$  is increased with increased alkalinity of the urine. Just as  $\text{NH}_4^+$  serves to excrete anions without loss of mineral cations, so  $\text{HCO}_3^-$  combines with the cations claiming excretion and affords a ready means of excretion of excess cations without expenditure of mineral anions. There is a threefold mechanism which limits the alkalinity of urine, the pH of which is determined by the ratio of  $[\text{HCO}_3^-]$  to  $[\text{dissolved CO}_2]$ , as is that of other body fluids, according to the Henderson-Hasselbalch equation (see p. 275). The ratio of  $[\text{HCO}_3^-]$  to  $[\text{dissolved CO}_2]$  increases more slowly than the  $[\text{HCO}_3^-]$  alone; the  $[\text{dissolved CO}_2]$  may be increased; and in addition, above pH 8.0 carbonate as well as bicarbonate is formed in appreciable amounts. Voided

urine becomes more alkaline unless precautions are taken to prevent loss of  $\text{CO}_2$  and  $\text{NH}_4^+$  formation. The  $\text{CO}_2$  tension of the urine may vary from 40 to 200 mm., with an average of 67 mm.<sup>42</sup> At a maximal  $\text{CO}_2$  tension and maximal alkalinity the  $\text{HCO}_3^-$  can neutralize 400 meq. of cations/l.<sup>31</sup> Normally  $[\text{HCO}_3^-]$  is 2 meq./l., or less.

The  $[\text{HCO}_3^-]$  in blood plasma is related not only to acid-base equilibrium, but also to the  $\text{Ca}^{++}$  equilibrium.  $[\text{Ca}^{++}]$  is diminished when the  $[\text{HCO}_3^-]$  is increased. If  $[\text{HCO}_3^-]$  increases as a result of feeding alkali to patients with damaged kidneys, tetany supervenes. When  $\text{HCl}$  is lost by vomiting or obstruction,  $[\text{HCO}_3^-]$  increases in the blood serum and causes gastric tetany. The  $[\text{HCO}_3^-]$  is, however, only one of the factors affecting the ionization of calcium. Moreover, tetany may occur with hyperventilation in which the  $[\text{HCO}_3^-]$  is not increased, but the  $\text{CO}_2$  tension is diminished.

Bone salts contain calcium phosphate in combination with calcium carbonate as a complex salt, dahllite. Because of the ubiquitous nature of  $\text{HCO}_3^-$ , this ion is always available in adequate amounts for bone formation. This mechanism is described under *Calcification* (p. 140).

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## Chapter 6

### Calcium and Magnesium

Calcium is present in the body to a far greater extent than any of the other positive mineral elements. The requirements for both normal growth and maintenance constitute an important nutritional problem. The physico-chemical aspects of its action in the body have opened difficult questions, many as yet unanswered. It is not surprising therefore that the literature on calcium is probably as voluminous as that on all of the rest of the minerals together. The relations of calcium to phosphates, carbonates, magnesium, acid excretion, hormones and vitamins are particularly intimate and complex.

Description of many of its functions has been scattered throughout the book, and will not be repeated here. Many recent reviews are available.<sup>11, 21, 57, 75, 94, 146, 161, 177, 190</sup> In Chapter 2 a description of the distribution of calcium in the body shows that it is located almost exclusively in the skeleton. However, that small fraction not in the bones is of great physiological significance. Its relations to secretions and excretions are summarized in Chapter 3, to water metabolism in Chapter 12, to acid excretion in Chapter 13. The calcium intake, requirements, retentions and paths of excretion are discussed in Chapter 14. In addition to these aspects, calcium bears important relation to the coagulation of blood, lymph and milk, none of which is discussed.<sup>36</sup>

In this chapter are considered briefly the state of calcium in the blood, the mineralization of bone, pathological conditions related to calcium metabolism, and the role of calcium in neuromuscular irritability.

#### CALCIUM IN THE BLOOD

##### Calcium Content

The calcium in blood is found wholly or almost wholly in the serum. The red cell membrane is impermeable to calcium. Moreover, little hemoglobin combines with calcium even when serum is dialyzed against solutions of hemoglobin.<sup>154</sup> Several investigators have found that the true plasma contains about 1 mg./100 cc. more than serum, but this result has not been generally accepted.

The calcium content of serum is normally 10.0-10.5 mg./100 cc. (5.0-5.2 meq./l.). In infancy it is slightly higher, in the last months of preg-



nancy slightly lower,<sup>136, 141</sup> and is perhaps lower in extreme old age. The diurnal variation is small.<sup>35</sup>

**Factors affecting serum calcium.**—The main regulator of calcium in the blood is parathyroid hormone. The level of calcium in the serum rises or falls according to the amount of the hormone available. See further under *Parathyroid Glands* (p. 110), and *Rickets* (p. 143).

Vitamin D in excessive doses causes an elevation of the serum [Ca]. However its main effect is to maintain normal concentrations of calcium and phosphate. Whether the [Ca] in the blood is low and the [phosphate] high, or the [Ca] is at the upper limit of normal and the [phosphate] is low, the administration of vitamin D tends to alter the relationship toward normal. Long-continued low calcium intake in the absence of vitamin D causes decrease in blood [Ca].

Ingestion of calcium causes a rise so small that it was long overlooked. It has been shown<sup>161</sup> that 2 gm. of calcium, in the form of any of the soluble salts, causes an increase in the [Ca] of the serum of about 1.5 mg./100 cc. The rise reaches its maximum in about 2 hours and returns to normal in 4 hours. This increase is usually, but not necessarily, accompanied by an increase of inorganic phosphate concentration which reaches its maximum about 6 hours after the calcium ingestion.

Intravenous injection of calcium is followed by a rise in serum [Ca], and a drop in the [phosphate], although increases in the latter have been reported. Plasma [Mg] rises for a few hours.

Injection of either acid or alkaline phosphate in large amounts causes a decrease in serum [Ca]. The alkaline phosphate produces tetany.<sup>9</sup> When small amounts of phosphate are given there is a small rise in [Ca] and a subsequent fall below the initial level. But the [Mg] falls continuously.

Injections of soluble oxalates reduce the serum [Ca] to the point of convulsions or death. Citrates do not affect the total [Ca], but combine with  $\text{Ca}^{++}$  to form a complex ion, and tetany may supervene.

There is a relation between glucose and calcium as well as an effect of glucose on phosphorus metabolism (see p. 186). Both calcium and glucose have been used to control the bleeding in jaundice, for presumably they interact to increase the [Ca] of the blood, and hence to diminish the clotting time. However, when the serum [Ca] is raised to a considerable extent and over a long period of time, as in hyperparathyroidism, the clotting time is delayed.

### State of Calcium in Blood Serum

The state of calcium in the blood serum is quite different from that in simple inorganic solutions such as Ringer-Locke's, for the serum contains about three times as much calcium as can be held in an inorganic

solution of the same pH, which contains similar concentrations of phosphates, bicarbonate and other ions.

When serum is treated by dialysis against solutions of the same ionic strength but varying  $[Ca]$ , called compensation dialysis; or by ultrafiltration through collodion membranes; or by vividiffusion, that is, compensation dialysis with the return of the blood to the intact animal, some of the calcium is found in the dialysate, and some remains in the serum. Investigators are in agreement that two different states of calcium are thus differentiated. These fractions of calcium may be discussed under the following headings: non-diffusible, which includes proteinate and colloidal calcium; and diffusible, or dialyzable, which is related to ionic calcium.

**Non-diffusible calcium.—Proteinate.**—That protein bears an important relation to the calcium content of the blood is obvious from the observation that the amount of calcium in the various body fluids varies with the protein content. The blood serum contains 10 mg. of  $Ca/100$  cc., whereas the protein-free cerebrospinal fluid contains only 5 mg. Similarly other fluids, such as transudates and exudates, contain calcium in proportion to their protein content.<sup>107</sup> The amniotic fluid, and secretions such as milk, form separate problems. The relation of protein is further evident from studies in nephrosis and uremia, in which it has been shown that the calcium diminishes when the serum protein is decreased.<sup>159</sup>

When serum is electrolyzed some of the calcium migrates to the anode.<sup>7</sup> This probably represents a combination with protein to form a negatively charged ion. However, Greenberg and Greenberg<sup>44</sup> were unable to find any such calcium in serum or ultrafiltrates under normal conditions or after parathyroid injections.

Peters and Eiserson<sup>145</sup> calculate that in 100 cc. of serum, 3.9 mg. of  $Ca$  are normally present as proteinate (for the 7 gm. of protein present). It is roughly this fraction, which normally represents about 50 per cent of the total  $Ca$ , that is non-dialyzable. The amount of proteinate is in part dependent upon the albumin/globulin ratio, for the  $[anion]$  of the former is 50 per cent greater than that of the latter.<sup>197</sup> But the total proteinate and calcium proteinate are not necessarily proportional. Normally albumin forms 70 per cent of the serum protein, but this varies in pathological conditions. Moreover there are two globulins which form calcium proteinate in different proportions.<sup>24, 54</sup>

Recent investigations have shown that the protein fractions called albumin and globulin each includes a group of entities.\* Attempts have been made<sup>52, 145</sup> to give equations which relate the  $[Ca]$  of the serum to the  $[protein]$  or to the  $[protein + phosphate]$ . Schmidt and Greenberg<sup>161</sup> have maintained that these equations do not possess validity

\*Dr. E. J. Cohn, personal communication.

and that, in the present state of knowledge, such attempts cannot be entirely successful. The  $[Ca]$  of the blood is not always proportional to the  $[protein]$ . The  $[Ca]$ , as well as the  $[phosphate]$ , tends to be higher in new-born children although the  $[protein]$  does not. In hyperparathyroidism or with vitamin D therapy, the  $[Ca]$  is raised without any increase of the serum  $[protein]$ . Conversely, the  $[Ca]$  is diminished in hypoparathyroidism and in some other forms of tetany without alteration of the  $[protein]$ .

That no important fraction of calcium in the serum is bound by the lipids was shown by the fact that ether-extracted serum showed only slightly lowered  $[Ca]$ .<sup>105</sup>

The hen presents a special case. The non-laying hen has a total serum  $[Ca]$  of 12 mg./100 cc., of which the non-dialyzable  $[Ca]$  is 4 mg. The laying hen has a total  $[Ca]$  of 20 mg./100 cc., of which the non-dialyzable fraction is 12 mg. Thus the whole difference lies in the non-dialyzable portion. There is also a rise in the non-ionized fraction of the phosphate.<sup>23, 103</sup> This is probably due to the serum vitellin which is greatly increased.<sup>46</sup>

*Colloidal calcium.*—Although most of the non-diffusible calcium in the blood is present as proteinate, colloidal complexes of calcium phosphate have been reported.<sup>5, 102</sup> Ordinarily the colloidal fraction is present in the serum only in minimal amounts or not at all. It is formed when either calcium or phosphate is added in large amounts, or when they are increased by parathyroid hormone or vitamin D. It disappears rapidly from the blood stream, is probably taken up by the reticulo-endothelial system, and may not be available for precipitation in the bone.\* Under the conditions in which the concentration of colloidal calcium phosphate is increased, metastatic calcification occurs most frequently.

*Diffusible calcium.*—A considerable literature is extant to show that 50-60 per cent of the blood serum calcium is dialyzable or ultrafiltrable. One must proceed with great caution in interpreting and evaluating the quantitative results of these experiments. The type of membrane, composition and volume of dialyzing solution, pH, temperature and presence of lipids, all affect the result.<sup>31, 139, 161, 190, 203</sup> Loeb<sup>106</sup> was able to show that all the calcium was dialyzed through collodion membranes when large volumes of 0.8-per cent NaCl solutions were used either for a long time, or at pH 2.5. Serum has been dialyzed against cerebrospinal fluid without showing any change.<sup>132</sup> The amount of diffusible calcium obtained by ultrafiltration and by vividiffusion are in very close agreement.<sup>49</sup>

All measures which raise the  $[Ca]$  of serum— $CaCl_2$  injection, vitamin

\*Dr. F. C. McLean, personal communication.

D and parathyroid hormone—raise both fractions.<sup>45, 185, 193</sup> The exact nature of the fractions increased and their distribution is the subject of active investigation at present.

When citrate is injected, not only the ionized calcium but also the Ca of calcium proteinate combine with it to form a complex calcium citrate ion. The total serum [Ca] is not altered. Calcium in such combination is rapidly excreted. If, however, the excretion is prevented by tying off the kidneys, and the oxidation of citrate hindered by tying off the liver, calcium is drawn from the body stores to replace the depleted fractions and the total blood [Ca] is increased.\*

Largely because of the increased serum [Ca] after parathyroid hormone dosage, Greenwald<sup>51</sup> suggested that part of the calcium is in the form of a citrate-like compound.<sup>94</sup> However, Greenberg and Greenberg<sup>44</sup> have advanced evidence that such compounds are not present in serum. Further, after parathyroidectomy (and hence, presumably after the removal of such compounds) the serum is still capable of holding a normal amount of calcium.

Klinke<sup>92</sup> found that part of the calcium of serum and ultrafiltrates was adsorbed by  $\text{BaSO}_4$  and  $\text{Ca}_3(\text{PO}_4)_2$  which are positively charged, but not by kaolin, the particles of which are negatively charged. Benjamin and Hess<sup>6</sup> divided the total calcium into (1) protein-bound; (2) non-filtrable, adsorbable; (3) filtrable, adsorbable; (4) filtrable, non-adsorbable (ionic). This has not met with general acceptance because of the different values found according to the method of preparation of the adsorbent, and the varying electrical charge it carries. Hence adsorbable calcium cannot be considered as an entity. It is simplest to conceive that all the dialyzable calcium, according to modern theories, may be called ionized.

**Ionization of calcium.**—Even in simple salt solutions there is considerable doubt as to the degree of activity of calcium. When other cations and anions are present, the difficulties of interpretation are at present insurmountable. Several types of methods of measuring calcium activity have been developed.

**Calcium electrode.**—The most direct and specific method is that of the calcium electrode.<sup>137</sup> Recent studies of this have shown it to be unreliable in the presence of protein.<sup>161</sup>

**Colorimetric methods.**—Attempts have been made to use colored  $[\text{Ca}^{++}]$  indicators similar to those used for measurement of  $[\text{H}^+]$ .<sup>58</sup> These compounds are also affected by other ions and proteins and their application to biological systems has never been developed.

**Equilibration.**—If the calcium in the serum is in equilibrium with a slightly soluble calcium salt it should be possible to calculate the  $[\text{Ca}^{++}]$

\* Dr. A. B. Hastings, personal communication.

from the solubility product equations. If more calcium than this is present it must be in non-ionized form. Without going into a discussion of the physical chemistry, according to the old theory of solutions, there must be both ionized and non-ionized calcium in any solution of calcium salts. If, according to the activity theory, the calcium is regarded as completely ionized, the degree of activation, or the activity coefficient, is less than 1. Simply,  $\text{Ca}^{++} + \text{X}^{--} \rightarrow \text{CaX}$ ; or, stated according to the mass law:

$$[\text{Ca}^{++}] \times [\text{X}^{--}] = Ksp$$

in which  $Ksp$  is the solubility product. In this equation  $Ksp$  is not a constant but varies with the ionic strength of the solution. An attempt to use this principle has been made by Brinkman and Van Dam.<sup>16</sup> They added varying amounts of oxalate to blood serum, and calculated the solubility product from the first visible turbidity due to the precipitation of calcium oxalate. It has been shown, however, that owing to conditions of supersaturation, this method gives uncertain and inconstant results.

The definite relation between carbonates and the ionization of calcium was first developed by Rona and Takahashi<sup>155</sup> from Böldander, and has since been extended by Warburg.<sup>200</sup> The equation may be stated in terms similar to that above:

$$[\text{Ca}^{++}] = K \frac{[\text{H}^+]}{[\text{HCO}_3^-]}, \text{ in which } K = \frac{Ksp}{K_2}$$

In this equation  $K_2$  is the value of the second dissociation constant of  $\text{H}_2\text{CO}_3$ . This implies a system in equilibrium with solid  $\text{CaCO}_3$ . Such conditions are not present in the serum, which is undersaturated with regard to  $\text{CaCO}_3$ .

A great deal of work has, however, grown out of this method of approach. Attempts have been made to develop equations showing the interdependence of the  $[\text{Ca}^{++}]$  and the phosphate.<sup>38, 39, 71, 78, 94, 102</sup>

$$[\text{Ca}^{++}]^3 \times [\text{PO}_4^{---}]^2 = Ksp$$

In this equation the value of the  $[\text{PO}_4^{---}]$  strictly speaking is dependent upon and calculable from the total inorganic phosphorus, the three dissociation constants of phosphoric acid and the  $[\text{H}^+]$ ; however,  $\text{H}_3\text{PO}_4$  and  $\text{H}_2\text{PO}_4^-$  are present in negligible concentrations. This implies a system in equilibrium with solid  $\text{Ca}_3(\text{PO}_4)_2$ , which salt probably does not exist as such.

Important as these are in showing the relation of  $[\text{Ca}^{++}]$  to the formation of bone (see *Calcification*), it has not been shown that, under normal conditions, it is the concentrations of phosphates or carbonates which actually control  $[\text{Ca}^{++}]$ .

These equations have been improved by taking into account the effect of varying ionic strength (one-half of the sum of the concentrations of the ions raised to the square of the valence or charge of each

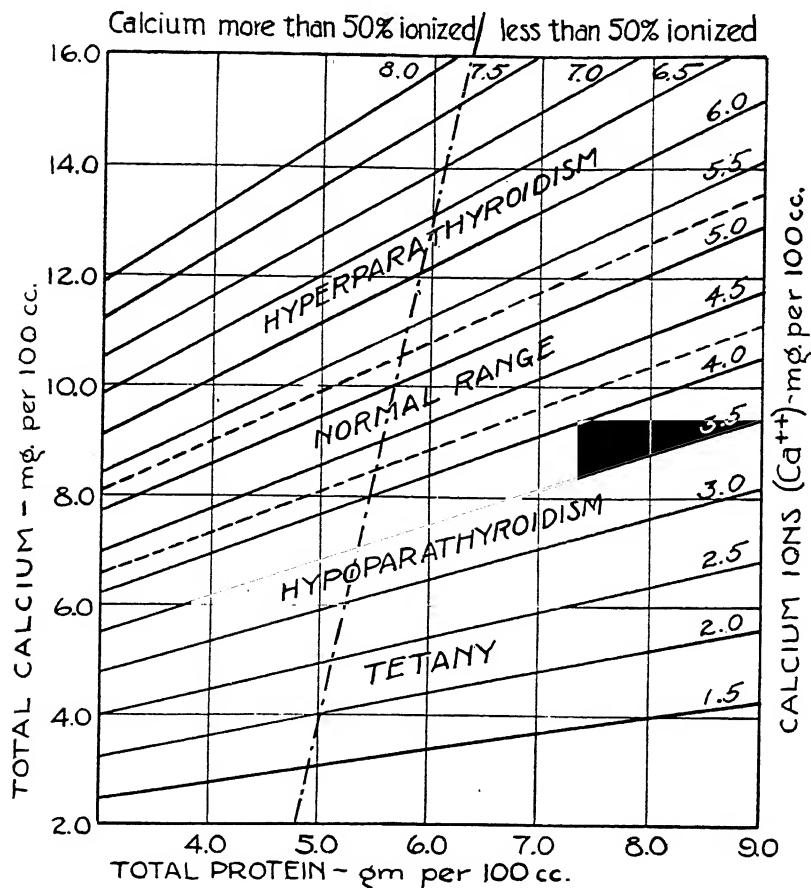


FIGURE 10. Chart for Calculation of  $[\text{Ca}^{++}]$  from Total Protein and Total Calcium of Serum or Plasma.\*

\* Figure 2 of McLean and Hastings,<sup>119a</sup> reproduced by permission of the American Journal of Medical Sciences.

ion) on the  $K_{sp}$ , according to the newer theory of solutions. Klinker<sup>94</sup> and Heubner<sup>75</sup> have further expressed these equations in terms of chemical thermodynamics.

From these general considerations it can be deduced that the  $[\text{Ca}^{++}]$  is inversely proportional to the concentration of anions which form

slightly soluble salts, and that it is increased with increase of  $[H^+]$  and of ionic strength.

*Calcium-protein equilibrium.*—Recently McLean and Hastings<sup>71, 118, 119</sup> have shown that the main determinant of  $[Ca^{++}]$  is the protein of the serum, according to the equation:

$$\frac{[Ca^{++}] \times [protein^-]}{[calcium\ proteinate]} = K$$

To determine the value of this  $K$  in serum they used the frog-heart method. This consists of comparison of the amplitude of the beat in the test solution with that in known solutions of  $CaCl_2$ . It was found that, in the serum,  $K = 10^{-2.22}$ . Thus the  $[Ca^{++}]$  can be calculated from the total  $[Ca]$  and the  $[protein]$ . All the dialyzable calcium is considered ionized, because the activity of this fraction is similar to that of inorganic solutions. The exact value of the activity coefficient has not been determined. From the nomogram of McLean and Hastings<sup>119a</sup> given in Figure 10, one may readily calculate the  $[Ca^{++}]$  in any sample of serum if the total  $[Ca]$  and the  $[protein]$  are known, and a pH of 7.35 is assumed.

In any mixture of electrolytes the  $[Ca^{++}]$  like the  $[H^+]$  is dependent upon the proportions of the various salts, and their solubility product constants. The buffering ions are limited by the concentration of ions which can be present without precipitation. This involves the  $K_{sp}$ , which in turn depends upon the nature of the substrate. The amounts of the buffer salts are further dependent on the acidity, which involves the pH and pK. These equations are so complicated that they have never been worked out in general terms. It is obvious however, from the work of McLean and Hastings, that the protein in the serum is the main determinant in the system which contains also  $HCO_3^-$ ,  $PO_4^{---}$  and  $HPO_4^{--}$ . Under these conditions it can be seen why the phosphate may vary so widely at any  $[Ca^{++}]$ .

## CALCIFICATION

### Mineral Composition of Bone

The idea that bone is an inert mass of lime salts is rapidly giving way to newer knowledge. Bone is a vascular structure, and the minerals may be readily removed by the action of the osteoclasts or bone destroying cells and the ions transferred to the blood. Bauer, Aub and Albright<sup>3</sup> have shown that the bone trabeculae increase and decrease, and can thus store or yield calcium and phosphate as the need arises. Acid diet, acidosis, excess parathyroid hormone and excess irradiated ergosterol all draw minerals from the bones. During pregnancy and lactation these sources are drawn upon and are later renewed. Such mobilization takes

place not only under stress, but is a constant daily occurrence. Aub and Calhoun \* have shown further, from studies with both madder root and radium, that material originally deposited almost wholly in the trabeculae is gradually disseminated. Radium over the course of years finally becomes evenly distributed throughout the bone shaft, which could happen only by the dissolution and rearrangement of the material in the trabeculae.

The nature of the composition of bone was discussed in Chapter 2. There it was shown that the minerals in bone can best be described as a complex apatite, similar to the mineral, dahllite. There is a nucleus of  $\text{Ca}_3(\text{PO}_4)_2$  upon which is adsorbed principally  $\text{CaCO}_3$ , but in addition,  $\text{CaHPO}_4$  and  $\text{Ca}(\text{OH})_2$ . The formula for this is  $n\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}$ , in which  $n$  has a value of between 2 and 3.<sup>83</sup>

**Changes in composition.**—The mineral composition of bone is nearly but not quite constant. No gross changes in the bones of chickens or rats could be demonstrated with widely divergent ratios of Ca/P in the food. In rickets the ratio of  $\text{PO}_4/\text{CO}_3$  is decreased;<sup>82</sup> on very low mineral intake with moderate phosphorus, the ratio of  $\text{PO}_4/\text{CO}_3$  is increased.<sup>19</sup> In old age the bones become more calcareous—richer in  $\text{CO}_3$  and poorer in  $\text{PO}_4$ . Acid poisoning removes more carbonate than phosphate from bone.<sup>25</sup> The small variation is due to the mechanism by which the blood stabilizes the calcium and phosphate regardless of the intake.

Primary calcification in cartilage has a different composition from bone.<sup>98</sup> After subtracting the calcium present as carbonate, the residual Ca/P ratio was found to be 2.23 in primary calcification, 2.01 in bone (1.94 in  $\text{Ca}_3(\text{PO}_4)_2$ ).<sup>98, 130</sup>

### Mineralization of Bone

The term calcification is used for the deposition of minerals in bone. This is unfortunate, as it does not take into account the minerals other than calcium. Because phosphate is often the limiting factor it would sometimes be more appropriate to speak of the phosphatification of bone. The term ossification, which is without specific implication, has not received general usage.

**Factors affecting calcification.**—Calcification includes more than the precipitation of carbonates and phosphates. It involves deposition in an organic matrix. This takes place at a definite site, the provisional zone of calcification. This is true not only of normal bones, but also of bone sections *in vitro*.<sup>175</sup> The normal growth of the cartilage cell, its degeneration, and the penetration of blood vessels in close approximation to the osteoid are fundamental to the process.

**Phosphatase.**—Robison<sup>148</sup> has demonstrated the presence in the body

\* Drs. J. C. Aub and K. A. Calhoun, unpublished data.



of enzymes which he calls phosphatases. These are capable of splitting the various phosphoric esters. An agent which leads to production of inorganic phosphate from organic phosphorus must be of considerable importance in the transfer of phosphorus across membranes and its precipitation with calcium.

Phosphatase occurs in maximal concentration at the site of calcification of bone. It is absent from that type of cartilage which does not calcify. Calcification *in vitro* is inhibited when phosphatase is not present. It is found not only in bone, but in comparatively large amounts in the kidneys and intestinal mucosa. Thus it occurs in the organs which compete for calcium and phosphate for deposition and excretion. Its role in the excretion of these substances has not yet been disclosed. It is present also in the blood vessels, which together with the kidney and intestinal tract are subject to metastatic calcification. The phosphatases together with other enzymes have been shown to play an essential part in the fermentations such as take place in yeast, or in the reactions in muscle activity. Phosphatase is not inactivated by fluoride or iodoacetic acid. (See Chapter 7, *Phosphorus*.)

Phosphatase occurs in small amount in the blood serum, but its role here is obscure. Kay<sup>88</sup> has interpreted its presence as a leakage from tissues of higher content. Variations in the blood phosphatase concentration are discussed under *Rickets*.

*Local factors.*—The importance of phosphatase is undoubted, but even this may not completely determine the whole of the local factor. It has not been demonstrated that phosphoric esters which can be split are actually present at the site of calcification. Calcium is however available, for the proteins of cartilage have been shown to act as stronger acids than other body proteins, and to be ionized to a greater extent. Freudenberg and György<sup>39</sup> have claimed that cartilage combines with ionized calcium, which is subsequently precipitated as carbonate and phosphate. Klinker<sup>92</sup> has advanced the thesis that calcium carbonate forms a complex with cartilage, before precipitation. Benjamin<sup>5</sup> has demonstrated that cartilage from the provisional zone of calcification, either normal or ricketic, removes from serum or serum ultrafiltrates, or inorganic solutions containing  $\text{Ca}^{++}$ ,  $\text{PO}_4^{---}$  and  $\text{CO}_3^{--}$ , portions of calcium, phosphorus and possibly carbonate similar to those removed by  $\text{BaSO}_4$ .

*Other factors.*—Magnesium has been shown to prevent calcification *in vitro*.<sup>130, 170</sup> Sugar has also been found to inhibit calcification.<sup>129</sup> Amino-acids and fats in physiological concentrations are unimportant, but the possible effects of fatty acids on calcification warrant further study.

The recent studies in scurvy leave no doubt that calcification of bones and teeth is dependent in part upon vitamin C or some other substance

present in orange juice.<sup>84, 79, 157</sup> Without this vitamin the osteoblasts, or bone-forming cells, do not develop normally.

The main factors which control calcification under physiological conditions are vitamin D and parathyroid hormone and the amounts and proportions of the ingested calcium and phosphorus. These factors are discussed under *Rickets*, *Tetany*, *Parathyroid Glands* and also in Chapter 14.

**Relation to calcium and phosphorus in blood serum.**—Before beginning a discussion of the conditions in the blood which cause precipitation in the bones, it is worth while to recall that the physiological mechanisms of the body tend to maintain the constancy of the concentrations of the circulating minerals. Just as, in anion-cation economy, large differences may be effected without great change in the acid-base equilibrium, so too, changes in calcium and phosphorus metabolism occur without great fluctuations in the concentrations of these elements in the blood serum.

The factors which govern absorption of minerals into the blood stream and those which control their removal act simultaneously. The blood draws its calcium and phosphate from either the food ingested or the stores in the skeleton. Examination of the blood serum does not enable one to judge from which source the minerals are derived. Thus, either calcification or decalcification may be taking place without great changes in concentrations in the blood. Withdrawal of calcium and phosphate from the blood is accomplished by competing mechanisms in the kidneys, intestines, tissues and bones. If colloidal or non-ionized calcium is removed by the reticulo-endothelial system, ionized calcium by the tissues, kidneys and intestines, and insoluble soaps by the intestines, each at a variable threshold, the problem becomes very complex. An exact evaluation of these simultaneous actions is not available in our present state of knowledge.

Whatever may be the ultimate determinant of precipitation of minerals in the bone matrix, the process of calcification depends primarily upon the calcium and phosphate in the serum. This was proved when bone was calcified in inorganic salt solutions *in vitro*.<sup>175</sup> But the mechanisms are at present only partially understood. Certainly the explanation by Howland and Kramer,<sup>80</sup> based on the product  $[Ca] \times [P]$ , is too simple to form a scientific basis; but the more one becomes involved in a consideration of the state of calcium and phosphorus in body fluids, the more difficult does an adequate explanation appear.

Studies of the solubility products of  $CaCO_3$  and  $Ca_3(PO_4)_2$  have been undertaken with the hope that calcification could be expressed in terms of the  $[Ca^{++}]$ ,  $[CO_3^{--}]$ , and  $[PO_4^{---}]$  in the serum.<sup>71, 78, 91, 98, 102, 165, 166, 167</sup> In general the serum was found to be undersaturated with regard to  $CaCO_3$ . When equilibrated with  $Ca_3(PO_4)_2$ , or bone, both calcium phosphate and carbonate were found to precipitate. Shear, Washburn

and Kramer<sup>167</sup> have suggested that the serum may be in equilibrium, not with  $\text{Ca}_3(\text{PO}_4)_2$ , but with  $\text{CaHPO}_4$ , in its primary calcification. Holt, La Mer and Chown<sup>78</sup> concluded that the serum was supersaturated not only normally, but even in rickets.

Recent studies by Logan and Taylor<sup>109, 110</sup> have done much to clarify the situation. They have shown that, when the solubility product of  $[\text{Ca}^{++}]^3 \times [\text{PO}_4^{---}]^2$  is exceeded in inorganic salt solutions,  $\text{Ca}_3(\text{PO}_4)_2$  precipitates. There is rapidly adsorbed upon this nucleus  $\text{CaCO}_3$ ,  $\text{CaHPO}_4$  or  $\text{Ca}(\text{OH})_2$ , according to the amounts available in the solution, even though these do not exceed the solubility product. This precipitate then gradually alters its composition over a period of weeks. Precipitation will continue at lower concentrations of the ions than were required to initiate it. The concentrations necessary for precipitation thus form a zone, rather than a definite point. Further, one of the factors affecting this variability is the amount of substrate present. It is thus evident that the chemistry of the solubility product of apatite is extremely complex, and quite unlike that of simpler slightly soluble salts. This explanation seems to the writer more satisfactory than the theory of permanent supersaturation of serum with respect to calcium and phosphate, or those of adsorption of  $\text{Ca}^{++}$  and  $\text{CaCO}_3$  upon cartilage.

Knowledge of the chemistry of calcification is in quite a different state from that of acid-base equilibrium. There the major factors are known quantitatively, and the effects of the carbonate and phosphate equilibria can be evaluated by a measurement of the  $[\text{H}^+]$ . Until calcium can be similarly quantitated, any explanation will fail to carry conviction. In all calcium equilibria of biological application, the acidity is of fundamental importance, but this applies to the pH at the site of precipitation, at or within the cartilage cell. This pH remains unknown. That hydrolysis of phosphate from organic phosphorus is important is very probable, but its exact relation to bone precipitation is unknown. The part played by magnesium, sodium, potassium and phosphorus increases the uncertainty of the exact value of the constants involved in equilibria experiments. It would be reasonable if the answer were found that the activities of calcium, phosphate and carbonate lie close to the point of saturation in the normal adult, exceed the solubility product in infancy, and fall below the point at which precipitation is possible in rickets.

**Fractures.**—Fractures illustrate the mechanism of calcification under stress. Shortly after the discovery of experimental rickets it was shown that absence of vitamin D might be a cause of un-united fractures. But under usual conditions in normal adults vitamin D has been found to be unimportant. So too has treatment with parathyroid extract. Bones heal normally in the absence of parathyroid glands, provided that the normal level of serum  $[\text{Ca}]$  is maintained. Increases in serum [phos-

phate], [Ca] and [phosphatase] during healing have been reported and denied. The new bone laid down has a high calcium content compared to phosphate, as does new bone laid down at the epiphyses. Diet has not been shown to be important. Evidently the extra burden of a fracture is not a sufficiently severe strain on the normal mechanism to be illuminating in the average case. Spontaneous fractures in rickets or hyperparathyroidism occur, but here the cause must be referred to the underlying condition.

**Teeth.**—It has been generally accepted that after teeth have developed they form a rigid, fixed structure. Recent experiments by Aub and Calhoun \* have shown, by the ingenious use of radium poisoning, that teeth should not be regarded as inert structures. Radium is deposited apparently only where calcium is deposited, and the deposition of ingested radium in the dentine thus indicates an active metabolic organ.

Much has been written about the relation of dental disease to diet. The literature is so contradictory concerning both experimental animals and man that credence is difficult. One school says that lack of vitamin D is the cause of dental disease; another states that it is due to vitamin C deficiency. Heredity and hygiene have been blamed. Infection and specific bacterial flora have been held responsible. Still other groups hold that the acid condition of the mouth or of the saliva, or that the acid-base equilibrium of the blood is the important factor. The chemical composition of saliva, especially with regard to phosphorus, has been held to be important. Carbohydrates and cereals (anti-vitamin D) and quality or quantity of protein have been indicted. Some hold that good teeth result only when all dietary factors are balanced and abundant. The use of soft and finely divided foods in the dietaries of modern civilized peoples is said to preclude the mastication necessary for healthy teeth.

*Dental caries* is a disease which first occurs as a break in the enamel, with softening of the underlying dentine. The condition is at first recognizable by means of a dental probe; it may, however, progress to form increasingly large cavities, or the process may be arrested. It appears most frequently after the age of infancy, especially in growing children, in adolescents, and in child-bearing women.

Klein and McCollum <sup>90</sup> have stated that deficiency of phosphorus in the diet is an important condition in susceptibility to, but not in the production of, dental caries in rats. Experiments on various types of animals fail to carry conviction, for in this field, as in others, results with one species cannot be carried over directly to another. Further, dental defects in experimental animals and human caries may not be

\* Drs. J. C. Aub and K. A. Calhoun, unpublished data.

similar. Essentially, however, tooth defects, as bone defects, are fundamentally related to calcium and phosphorus metabolism.

Children with carious teeth showed smaller balances of both calcium and phosphorus than those whose teeth were normal, or in which caries was arrested.<sup>15</sup> Mellanby<sup>123, 124, 125</sup> reported excellent results in preventing the spread of caries in children by addition of vitamin D and reduction of cereal intakes. She accepts as proved that vitamin D is the *sine qua non* of sound teeth. The studies of Hanke<sup>65</sup> demonstrate clearly that a pint of orange juice daily not only increases the growth of children, but definitely improves the condition of the gums, and arrests dental caries.

Careful critical studies of the relation of rickets to dental caries have been made.<sup>34, 50, 73</sup> All agree that negro children have more and worse rickets and less dental caries, but more hypoplastic defects than white children. There is some correlation between rickets and caries in the deciduous teeth; the incidence of caries in the permanent teeth of ricketic and non-ricketic children is approximately the same. Tetany and serious infectious diseases are probably more important factors.

McCarrison<sup>115</sup> states that the tropical sun of India does not prevent caries. Eliot and Jackson<sup>33</sup> found dental caries, but no rickets in Puerto Rico. Caries is a world-wide problem, for it occurs not only in oatmeal-eating Scotland, but also in Hawaii, the land of sunshine and fruits. When one considers that caries may not occur in defective teeth, and may occur in sound teeth; that dirty mouths may be free, and "clean" mouths carious; and that so many alternative cures are strongly advocated, one is forced to the conclusion that the solution has not yet been reached. The recent work on dental caries has been excellently reviewed by Koehne.<sup>95, 96</sup>

Fluorosis is described under *Fluorine* (p. 243). The special problems of pregnancy and lactation are discussed on page 341.

### Rickets

More has been learned about rickets in the last two decades than in the three centuries following the description of the condition by Glisson. When Mellanby<sup>121</sup> produced rickets experimentally in dogs, and focused attention on the fat-soluble vitamins, a new chapter in calcium metabolism was opened. The successful production of rickets in rats, and its cure with vitamin D by Sherman and Pappenheimer<sup>173</sup> and by McCollum *et al.*<sup>116</sup> led to rapid developments. Much that has been learned of the etiology, pathogenesis and therapy of experimental rickets has been applied to the condition in humans. The clinical demonstration of the therapeutic effect of light by Huldchinsky,<sup>84</sup> and the discovery by Hess and Weinstock<sup>74</sup> and Steenbock and Black<sup>188</sup> that ultraviolet light ren-

ders ergosterol antiricketic, were so remarkable that the general public has become vitamin D-conscious.

Exhaustive reviews have been made by György,<sup>56</sup> Hess<sup>72</sup> and Goldblatt.<sup>42</sup> Recent literature has been reviewed by Harris.<sup>67</sup> So much has been done in this field that sheds light upon the mechanism of calcification that discussion of some of the data is justified.

The disease usually occurs from the 6th to the 18th month of age, during the period of most rapid bone growth, though occasionally later; in adolescence and mature life it is called osteomalacia. Although many other factors are of importance, the defect is due primarily to lack of exposure to sunlight, or to deficiency of vitamin D in the diet. The lesions heal and the underlying condition is corrected when adequate amounts of vitamin D are given, or the patient is irradiated with ultra-violet light.

**Bone lesions.**—Rickets is a general disease, but most interest has centered upon the lesions in the bones. The proliferating cartilage fails to ossify at the provisional zone of calcification, and large ricketic metaphyses result. These changes are typified by the gross manifestations which we recognize clinically—enlargement of the wrists, knees and ankles, beading of the ribs (the ricketic rosary), Harrison's groove (contraction of the chest), craniotabes (thinning) or square head (thickening of the bones of the head), bow legs and malformation of the spine; from this last symptom the name of the disease is derived. There are four principal methods of studying the bone lesions in rickets, all of which give comparable results.

*Histological examination* reveals a characteristic pathology. This is typified by an increased number of cartilage cells which no longer show their short columnar arrangement, sharp edge and clear degenerated cells at the line of calcification. Large amounts of cartilage and osteoid tissue are carried down into the shaft of the bone. This method of study is the best available for differentiation of rickets from other pathological bone conditions, and for detection of early lesions or the beginning of healing. However it does not give a quantitative evaluation of the severity of the disease.

*X-rays* have been of great service in the study of rickets. Roentgenograms disclose both the extent of the ricketic metaphysis and the presence and amount of calcification, and serve as the main criterion in clinical studies. This method has been used extensively in both animal experiments and vitamin D testing.

The *line test* of McCollum *et al.*<sup>117</sup> consists of staining the split bone with silver nitrate to disclose the line of new calcification.

*Chemical analysis* of the bones for ash, water, fat and organic matter gives further information. In rickets the amount of minerals laid down is always greatly diminished, and the water, fat and organic matter are

proportionally greater than in normals. An increase in the ratio of  $\text{CO}_3/\text{PO}_4$  in rat bones has been shown.<sup>82</sup> Other differences in chemical composition between normal and ricketic bones are much more marked in experimental animals than in infants.

Table 13.—Composition of Ribs of Normal and Ricketic Infants.\*

Substance	Normal Infant † (%)	Ricketic Infant ‡ (%)
Water .....	11.56	10.70
Organic matter .....	37.14	42.73
Sodium .....	0.64	0.73
Potassium .....	0.30	0.31
Calcium .....	24.40	21.47
Magnesium .....	0.10	0.63
Phosphate ( $\text{PO}_4$ ) .....	33.56	30.38
Phosphorus .....	10.93	9.90
Carbonate .....	3.11	2.70

\* Gassmann.<sup>41</sup>

† The normal subject was a boy 2½ years old.

‡ The ricketic subject was a boy 1½ years old.

The data of Gassmann,<sup>41</sup> given in Table 13, give a comparison of the bones of ricketic infants with those of normals. They show, in rickets, a lower Ca,  $\text{PO}_4$  and  $\text{CO}_3$  content, and a higher Mg content; due to the increased interstitial water, more  $\text{Na}^+$ ,  $\text{Cl}^-$  and more organic material are present, per unit of weight.

**Growth.**—Body growth and development are coördinated with, and limited by, skeletal growth. Paucity of minerals necessary for the formation of bones retards the development not only of the skeleton, but of the whole body. It is quite true that a certain amount of growth can take place with deficient mineralization of the bone: in fact most clinicians are agreed that the more rapidly the child grows, the more severe is the rickets. However, this is not true in experimental rickets; here growth is always subnormal, and advanced rickets may be produced coincidentally with weight losses. In such states it is not unusual to find a ricketic bone of half the weight of the corresponding bone of a normal animal of the same age. If the bone growth is inadequate, in the long run the body growth is always retarded. This end result applies not only to experimental rickets, but also to that of infants. Long-continued undermineralization leads to a condition called ricketic dwarfism.

**Blood.**—The  $[\text{Ca}]$  in the blood serum in rickets is usually found to be normal or slightly increased. The distribution of the various fractions, ionized and non-ionized, is not altered. The inorganic phosphate content (normally 5-6 mg./100 cc. in the serum of infants) falls below 3, and sometimes to 1 mg./100 cc. of serum. The glycerophosphoric acid frac-

tion in the red cells has recently been found to be markedly lowered.\*<sup>187</sup> The ester phosphorus content of the red blood cells is diminished in experimental rickets also.<sup>87</sup>

There is a form of rickets seen in premature infants in which both the [Ca] and [phosphate] of the serum are low. Often the common form of rickets shows low calcium-high phosphate values in the serum during recovery, especially if the therapy has been inadequate or interrupted. The same is true in experimental rickets when food is withheld, or when moderate amounts of phosphate are given. Under such conditions the blood serum values of calcium and phosphate are particularly unstable; tetany often supervenes. (See further under *Tetany*.) Clinical rickets in which the [phosphate] is not low, or in which healing occurs without increase<sup>186</sup> has rarely been reported.

When experimental rickets is produced with varying ratios of Ca/P in the diet, the blood reflects this ratio, so that it is possible to have either high calcium-low phosphate, or low calcium-high phosphate values in the serum associated with rickets.<sup>97</sup> With increasing levels of intake both the [Ca] and [phosphate] in serum tend to rise. The reason that the blood findings are so constant in clinical rickets is that infants receive modified milk diets, and therefore a constant proportion of calcium to phosphorus.

*Phosphatase*.—The [phosphatase] in blood serum is found to be increased not only in rickets, but in other generalized bone diseases in which osteoblastic activity is increased. This rise is one of the earliest manifestations of the disease process, and the return to normal one of the latest of the cure of these conditions. The increase seems to be a compensatory mechanism for the precipitation of minerals in the bone.<sup>13, 134</sup>

*Intake*.—The single defect necessary to produce rickets in human beings is an absence of vitamin D, but in the rat an imbalance of calcium and phosphorus in the diet has been considered essential. In this respect the rickets of dogs more nearly approaches that of infants than does that of the rat.

*Ca/P ratio*.—McCollum *et al.*<sup>116</sup> and Sherman and Pappenheimer<sup>173</sup> produced rickets in rats with diets relatively high in calcium and low in phosphorus. (A second type of rickets produced by very low calcium and moderate phosphorus levels in the diet has been investigated so little that we shall not discuss it.) The investigators were eager to obtain a lesion so marked and so constant that it would be beyond dispute. Therefore they used diets in which the Ca/P ratio varied from 6/1 to 4/1. At ratios as low as 3/1 and 2.5/1, moderate rickets is still pro-

\* Drs. G. M. Guest and S. Rapoport, personal communication.



duced, although at 2/1 the bones are normal. Even this ratio is higher than that in milk, which is  $\text{Ca/P} = 1.3/1$ .

The importance of the ratio of  $\text{Ca/P}$  is undoubted, but the total concentration or level must also be taken into account. If the level of the calcium and phosphorus is low enough, rickets can be produced in rats at the same, or even at a lower  $\text{Ca/P}$  ratio than that found in milk. As the total amounts increase at any given ratio, the diet becomes less ricketogenic. If sufficient amounts of calcium and phosphorus are included, diets with a  $\text{Ca/P}$  ratio of 6/1 are not ricketogenic.

Because both calcium and phosphorus are necessary to form bone, theoretically any substance which prevents the adequate retention of either should lead to rickets. Experiments with agents which interfere with the utilization of calcium are lacking. With low phosphorus diets not only does high calcium prevent adequate phosphorus utilization, but excesses of other metals which form insoluble phosphates (except barium, which is too toxic) also cause rickets. In this list are Be,<sup>55</sup> Mg,<sup>128, 135</sup> Sr,<sup>176</sup> Fe,<sup>17</sup> Pb<sup>168</sup> and Tl.<sup>153</sup>

The ricketogenic effects of calcium and phosphorus are quantitatively related to the amount of vitamin D administered.<sup>8</sup> The greater the imbalance of calcium and phosphorus, the greater is the amount of vitamin D required to prevent rickets in rats. It is difficult to transfer this experience to rickets in infants. The intake of infants is favorably constituted as to both ratios and levels of calcium and phosphorus. Cow's milk must be considered high in minerals, for breast milk contains only one-fourth as much calcium and phosphorus (and produces less rickets). It is obvious that no two species react exactly alike to the same dietary stimulus, and it has also been shown that chickens, rats and babies react differently to the various forms of vitamin D.

*Excess of negative minerals.*—It has been held that rickets is associated with excess anion production and tetany with excess cation production. No one has found a diminished pH of the blood in rickets; although some have found the  $[\text{CO}_2]$  reduced, others have reported it as normal. The  $\text{NH}_4^+$  in the urine is increased, and ricketic infants can consume more  $\text{NaHCO}_3$  than normals without rendering the urine alkaline; but these are not conclusive signs that the excretion is more acid than normal, for the titratable acidity of the urine is diminished.<sup>133</sup> Excretion of urine more acid than normal is undoubtedly associated with diminished deposition of minerals in bone. The ricketogenic diets commonly used for rats contain a large excess of positive mineral equivalents due to the  $\text{CaCO}_3$  content; a similar degree of rickets results when  $\text{CaCl}_2$ , which in metabolism is an acidogen, is substituted. But the great disproportion of the  $\text{Ca/P}$  ratio noted above masks the effect of excess of either positive or negative minerals. If diets with smaller disproportion are used, mild spontaneously healing rickets can be produced by acid

diets when neutral or alkaline diets of the same Ca/P ratio produce no rickets.<sup>179</sup>

It is known that ingestion of acid has a definite relation to absorption and utilization of calcium. If the contents of the intestinal tract are acid, calcium is more readily absorbed, but when excess anions are to be excreted, the calcium excretion is increased. The latter effect is more marked than the former, and the resultant is a diminished calcium retention. On the other hand, an excess of cations hinders absorption in the intestines, but facilitates precipitation in the bone, and hence diminishes calcium excretion. Partly neutralized organic acids constitute a buffer system which is acid when ingested; after absorption the organic radical is oxidized and an excess of mineral cations remains. Ingestion of such mixtures produces an acid pH in the intestinal tract and an excess of mineral cations for excretion. When animals were fed a ricketogenic diet supplemented with acid sodium tartrate or with citric acid plus sodium citrate, thus causing intestinal acidity and increased mineral cation excretion, rickets was completely prevented. Rickets was produced by feeding a non-ricketogenic diet to which was added ammonium carbonate plus ammonium chloride, thus causing intestinal alkalinity and increased mineral anion excretion.<sup>59, 60, 178</sup>

Shohl found that this effect was not due entirely to the anion-cation relationships, but also to a specific organic anion effect.<sup>178</sup> Of many organic anions tested, only the citrate, and to a lesser extent the tartrate, were effective. These results were obtained not only with a single ricketogenic diet, but with high, normal and low ratios and levels of calcium and phosphorus.

**Excretion.**—In clinical rickets there is an increased excretion of calcium in the feces, and the amount in the urine, normally small, is decreased. The phosphorus elimination in the feces is also markedly increased. As was first shown by Schabad,<sup>160</sup> it may exceed the fecal calcium. In infantile rickets, both the calcium and phosphorus balances are subnormal. They may be negative when the condition is severe, but such is not usually the case.

**Vitamin D.**—Vitamin D prevents or cures rickets. Until recently, irradiated ergosterol was the substance presumed to be the active agent either in natural products, or produced in animals or foods by irradiation with ultraviolet light. Now that the multiple nature of vitamin D has been established, a new chapter lies before us, as yet unwritten. That many new active substances will be found or synthesized now seems probable. The presence of several naturally occurring substances has been postulated from the different quantitative response by different species in the healing of rickets. It is not too much to expect that qualitative differences will also be shown.

The effect of vitamin D on rickets is quite striking. Not only are calcium and phosphate of the blood serum restored to their normal concentrations, but the metabolism also is markedly altered. The amount of calcium and phosphorus in the feces is reduced. That in the urine increases, and the retentions of both are increased.<sup>152</sup>

How vitamin D causes the healing of rickets is as yet not completely understood. Presumably it acts only through its effect upon calcium and phosphorus metabolism. There is no fundamental abnormality in the ricketic bone which prevents its calcification; slices of ricketic bone placed in normal serum or inorganic salt solutions become calcified.<sup>149, 150, 174, 175</sup> Further, it has been shown<sup>66</sup> *in vitro* that there is no reason to believe that vitamin D acts directly on the bone cells to cause calcification.

Recent work, especially that of Nicolayson,<sup>140</sup> indicates that vitamin D permits a greater absorption of calcium from the intestine, which in turn increases absorption of phosphate. This increased absorption maintains the normal concentrations of calcium and phosphate in the blood serum, and decreases parathyroid activity. Thus the withdrawal of minerals from the bones is arrested, and the flow of calcium and phosphate from the blood to the bones is restored.

**Parathyroid hormone.**—It was originally shown by Erdheim and has been repeatedly confirmed, that the parathyroid glands enlarge and their cells hypertrophy in rickets. An increased concentration of parathyroid hormone in the serum of ricketic rabbits has been demonstrated.<sup>61</sup> This overactivity of the parathyroid glands is not the cause of rickets, but is caused by rickets. This reaction is apparently a defense mechanism of the body to preserve the normal level of serum calcium so essential to the body economy. However, this hyperfunction of the parathyroids intensifies rather than ameliorates the ricketic condition. Injection of the hormone has been found to exaggerate the ricketic process in rats,<sup>28, 169, 198</sup> and to retard healing.<sup>37, 76</sup> It is more difficult to produce rickets after removal of the parathyroids.<sup>143, 169</sup> A. T. 10, like parathyroid hormone does not cure rickets. For further description of the interrelation of vitamin D, parathyroid hormone and A. T. 10 see *Parathyroid Glands*.

**Other factors.**—Although the intakes of calcium, phosphorus and vitamin D are the main factors in the etiology of rickets, the quality of protein and carbohydrate have also been indicted. The availability of calcium in different foods is mentioned in Chapter 14 (p. 348). It has been suggested that the phosphorus of casein is not as well utilized as other forms. It has been claimed by Mellanby<sup>122</sup> that cereals contain anti-calcifying substances, but it seems probable that this effect is due to the form in which phosphorus occurs (see Chapter 7, *Phosphorus*, p. 187).

Abnormal fat metabolism has a much more obvious relation to rickets. If fat is not utilized and fatty acids are excreted by the intestine,

they form insoluble calcium soaps, and thus cause a lowered calcium retention,<sup>77, 192</sup> In such conditions as fatty diarrhea, celiac disease and sprue, rickets and tetany may supervene.<sup>112, 144</sup>

Interference with normal absorption or excretion of calcium and phosphorus may result from damaged liver or kidneys. Such conditions have been described as hepatic rickets and renal rickets. The latter is often associated with high phosphate concentration in the blood serum, and is complicated by acidosis.

Certain rare cases of rickets have been reported which are resistant to vitamin D therapy, and require many thousand times the usual therapeutic dosage.

### Metastatic Calcification

Calcification in tissues was described nearly a century ago by Virchow and has been the cause of much speculation and experimentation since that time. The mineral composition of such material is similar to that of bones, but the ratio of Ca/P is usually somewhat increased. The sites where metastatic calcification occurs most commonly are the kidneys, stomach, lungs and arteries. These include the tissues from which the chief secretions of acid take place, thus strongly indicating an alkaline reaction in the tissue itself. Such a condition favors calcium phosphate deposition *in vitro*. Rabl<sup>147</sup> thought that if he could increase the [Ca] and [phosphate] in the serum and suddenly shift the pH toward the alkaline side, lime salts would be deposited. He fed mice a diet of milk made acid with phosphoric acid, alternating with milk plus sodium acetate plus potato. Definite metastatic calcification was thus produced, more so than with the acid phosphate diet alone. Alkaline phosphates by mouth did not induce the condition. However, alkaline phosphates parenterally have since been shown to be effective. Obviously then, the anion-cation relations are important, as they are in rickets. Acidity favors absorption, and alkalinity after absorption favors precipitation.

Whatever the effects of ingestion of acid and alkali may be, it is probable that they take place only when increased [Ca] or [phosphate] or both are present in the blood serum. The main causes of such a condition are excess vitamin D and parathyroid hormone.

*Hypervitaminosis-D.*—Very large amounts of vitamin D, of the order of 500-1000 times the therapeutic dose, cause death of experimental animals and persons. The severity of the lesions and the time of survival depend on the dosage. Toxic doses cause increases of serum [Ca] and [phosphate], and increased density of the bone at the site of calcification. With still larger amounts calcium and phosphate are withdrawn from bone, but calcification continues, so that calcium and phosphate may be withdrawn from the shaft and deposited at the epiphysis. If long continued, osteitis fibrosa is finally produced.

The other conditions which follow hypervitaminosis-D are loss of weight, diarrhea, negative calcium balances, and extensive calcification of the heart, arteries, kidney and stomach.<sup>68, 180</sup>

The relation of calcium and phosphorus intake to vitamin D is made clear in this condition, for with a ricketogenic diet the effects of excess vitamin D are not so marked as with a normal diet.

*Parathyroid hormone.*—Metastatic calcification is produced by excess parathyroid activity as well as by excess vitamin D.<sup>(2)</sup> Clinically it is seen as a result of parathyroid tumors. Differing from hypervitaminosis, the rarefaction and cystic formation of the bones is marked, but the type of abnormal calcium deposition has not been reported as essentially different. The condition is also associated with high [Ca] (but not with high [phosphate]) in the blood plasma. The effect is intensified by high calcium intake, and reduced by low calcium. Metastatic calcification is also induced by high phosphorus intake, the primary effect of which is to lower serum [Ca] by the precipitation of calcium phosphate. Acid administration intensifies the effect of excess hormone. The damage is increased by large intakes of vitamin D and lessened when the vitamin intake is deficient. The effect of vitamin D in toxic amounts has been reported as approximately the same whether or not the parathyroids were present. (See further under *Parathyroid Glands*.) Similar results can be obtained by giving toxic doses of A. T. 10.\*

The condition of metastatic calcification is thus seen to result from a high calcium intake, excess vitamin D, A. T. 10, or parathyroid hormone, and hence from prolonged elevation of serum [Ca], whatever the cause. These are the conditions shown previously to lead to formation of colloidal calcium phosphate in the blood. Its removal by the tissues creates nuclei on which, under continuing conditions, further precipitation takes place.

*Calcinosis universalis* is a rare condition in which the calcium deposits are subcutaneous, instead of in the organs in which metastatic calcification normally occurs. When the deposits are superficial and localized the name *calcinosis circumscripta* or calcium gout is given. The deposition takes place in fat tissue, and has a Ca/P ratio similar to that found in other pathological calcifications. The outstanding abnormality in metabolism is the increased retention of calcium and phosphorus, even on very low intake. The plasma [Ca] and [phosphate] are normal except following high calcium intake, when the [Ca] may rise to 15 mg./100 cc. Hence this type of abnormality cannot be classed under high or low parathyroid, thyroid, calcium, or vitamin D effects, and for the present, at least, must represent a hitherto undescribed factor in calcification.<sup>4, 40, 189</sup>

\* A. T. Shohl and C. Fan, unpublished data.

*Other forms* of metastatic calcification such as the important arteriosclerosis, often involve changes in the fat and cholesterol metabolism, which lie beyond our field of discussion. So too do those changes which occur after inflammation in muscles, and after necrosis of tissue, such as calcification in tuberculous caseous degeneration.

Cataract is a condition in which calcification occurs in the lens. It may be due to various causes: congenital, complication of tetany, vitamin B complex deficiency, excess lactose or galactose in the diet, or senile changes.<sup>14</sup>

### Urinary Calculi

Some stones may be found in the pelvis of the kidney, others which form smaller aggregates lodge in the ureter, those still smaller are passed as gravel either with or without symptoms. If the material is precipitated in the parenchyma it is called metastatic calcification.

Crystals of oxalate and phosphate of calcium and magnesium are an extremely common finding in normal urine. The majority of urinary calculi contain calcium, magnesium, phosphate, carbonate and organic matter in various proportions. Many stones are described with a center of one type, covered by calcium phosphate and urate in concentric layers. Stones which contain cystine may occur in the bladder. This rare metabolic anomaly has been mentioned in Chapter 8, *Sulfur*. Gallstones have been mentioned (Chapter 3) as consisting essentially of cholesterol and bile pigment. Both of these types also contain mixtures of calcium,  $\text{PO}_4$ , magnesium and  $\text{CO}_3$ . So too do the rare concretions found in other parts of the body, such as stones in the prostate and parotid glands.

Medical authorities have little to offer as to the cause of calculus formation. In addition to anatomical and infectious causes there must remain a large proportion to be explained on a physiological basis. The length of time for stone formation is known only roughly, but many exist for years without apparent growth. Experimentally stones have been produced by Keyser<sup>89</sup> by oxamid and butyl oxalate, but were not found following calcium or phosphate ingestion or injection. It is highly suggestive that vitamin A deprivation in the rat leads to phosphate stone formation in the kidney tubules, pelvis and ureters. McCarrison has suggested that stone in India is of such dietetic origin.<sup>114</sup> Many writers claim that nephrolithiasis follows fractures, especially compound, or other destructive bone diseases, such as osteomyelitis. One naturally thinks of the calcium and phosphate concentration in the diet, blood and urine as possible causative factors.

Renal rickets results in high serum [phosphate]; in children with this disease both [Ca] and [phosphate] may be increased. This condition is also frequently associated with kidney stones. Hyperparathyroidism

and hypervitaminosis-D are now known to be closely associated with increased excretion of calcium in the urine, and with stone formation. Hunter<sup>55</sup> found ten stones in 32 cases of proved hyperparathyroidism. Albright *et al.*<sup>1</sup> have recently found many stones to be associated with an increased serum [Ca], and the deduction is inescapable that in the future this correlation will assume greater importance, in proportion to the frequency with which it is sought.

The aim of therapeutics other than surgical has been to find a method of dissolving the stones or preventing their occurrence or recurrence. One of the principal points studied has been the acidity of the urine, the idea being that an acid urine dissolves calcium and magnesium phosphates, and an alkaline one precipitates these. Citrates have a special capacity for dissolving calcium salts. It is of interest therefore, that Albright *et al.*<sup>\*</sup> have found that some stones disappeared after long-continued administration of large amounts of citrates.

### TETANY

The problem of irritability of tissue in general is closely related to the minerals. Every cell is affected by its chemical environment—the muscle cell for contractility, the gland cell for secretion and the nerve cell for conductance. The physiology of taste involves, in addition to the state of the nerve, the chemical state of the sapid material. Both the ionic concentration and osmotic pressure are factors in determining the degree of stimulation. The rate and depth of respiration are governed nervously by the respiratory center, which in turn is regulated by the state of the blood, especially in regard to the  $[H^+]$ ,  $[HCO_3^-]$  and  $CO_2$  tension.

The condition of hyperirritability of the neuromuscular system is manifested by generalized convulsions, or by carpopedal spasm, laryngospasm, Chvostek sign (reflex contraction on mechanical stimulation of facial nerve) and Trousseau sign (carpal spasm on constriction of blood vessels of the arm). It is best measured by the galvanic current reactions of Erb. The amount of current necessary to cause contraction by opening (O) and closing (C) is in the ascending order for anodal (A) and cathodal (C) stimulation:  $CC < AC < AO < CO$ . The amperage required varies for different species, due to variable resistances, but the series is the same. The irritability increases from infancy to maturity. There may be anodal reversibility ( $AO < AC$ ) in tetany, but always the amount of stimulus required to produce contraction is reduced. In an infant, if the CO contraction requires less than five milliamperes, the diagnosis of infantile tetany is justified.

\* Dr. F. Albright *et al.*, unpublished data.

### Factors Affecting Irritability

That the irritability of the neuromuscular system is controlled by the ionic concentration has developed over the last thirty years, from the classic studies of Ringer, Loeb, Howell, and many others. It early became evident that increase of  $[K^+]$  in the fluid bathing the muscles causes increased irritability; calcium acts in just the opposite manner. However, because the  $[Ca]$  in the blood serum is rarely increased in life, it is more convenient to state that increase in  $[K^+]$  or diminution in  $[Ca]$  both cause increased irritability. A great deal has been written to show that the monovalent ions,  $Na^+$  and  $K^+$ , counteract many of the effects of  $Ca^{++}$  and  $Mg^{++}$ . It remained for Meltzer to add a new chapter to this branch of physiology when he showed that, when introduced parenterally,  $Mg^{++}$  and  $Ca^{++}$  were also antagonistic. The relation  $\frac{Na + K}{Ca + Mg}$  in the blood serum has often been used as a measurement of the electrolytes with regard to irritability. Obviously, if colloidal and non-ionized forms are present, such an expression cannot be valid. The acidity is also of importance. The relation may therefore be roughly stated in the pseudo-mathematical form:

$$\text{Irritability} \propto \frac{[Na^+] + [K^+]}{[Ca^{++}] + [Mg^{++}] + [H^+]}$$

It may thus be seen that there are five factors, any one of which causes a change in irritability. Because, however, the changes in  $[Na^+]$  and  $[K^+]$  under experimental or pathological conditions are so small, attention has been focused primarily upon  $[Ca^{++}]$ ,  $[H^+]$  and recently upon  $[Mg^{++}]$ . Of these the magnesium tetany, which is unknown chemically, can best be discussed under *Magnesium*.

Although the  $[H^+]$  and  $[Ca^{++}]$  in the body may govern the state of tetany, in our opinion the primary cause lies within the cells. Whether this is in the muscle cell or nerve cell, or at the junction of the two, is open to argument. Curare, which paralyzes the neuromuscular junction, will prevent tetany.<sup>69</sup> Hence the exciting cause is shown to lie in the nervous mechanism. But curare does not abolish the tetany caused by low  $[Mg^{++}]$ .<sup>48</sup>

Further, it is not implied that the contents of the cell reflect the concentrations outside the cell, for we have shown that the cell membrane is impervious to cations. Although no consistent changes within the cell have been shown in tetany, this does not mean that the cells are not sensitive to changes in their ionic environment. Sabbatani and his pupils<sup>156</sup> showed that when an isotonic solution of a calcium salt was applied to motor areas of the surface of the cerebrum, irritability to electrical stimulation was markedly diminished, as well as after intravenous



injection of calcium. On the other hand, salts which precipitate calcium, for example oxalates, brought about an increased irritability. How this mechanism works is unknown. Brain tissue has the ability to unite with calcium to a greater extent than any other except cartilage.

Certain non-ionized compounds may also affect the irritability of the cell. In fact, one theory of tetany, now not generally accepted, claimed that the presence of guanidine, and not the state of ionization, is the cause of clinical tetany.

Tetany may be associated with anoxemia. The exact mechanism of Trousseau's sign is not known, but it is presumably related to the lack of oxygen thus induced. Therefore the conclusion is inescapable that anoxemia in the tissues may be a predisposing or exciting cause of tetany.

Fever of any origin is also an inciting cause of tetany. An attack may be induced by a slight infection. Whether the increased temperature affects the ionic equilibria directly, or whether the increased rate of metabolism causes a greater tissue destruction and hence increased [phosphate] in the blood, is not known.

Although it cannot be definitely proved, it seems reasonable that the body cells are in equilibrium with the body fluids, and that the alteration of the [cation] of the fluids, especially of the blood plasma, is a valid measure of the state of neuromuscular irritability. All clinical forms of tetany bear some relation either to a diminished total  $[Ca]$  or  $[Ca^{++}]$  or to increased pH. It is impossible to define the various fractions of calcium in the blood in exact quantitative equations. It has not been shown that  $Ca^{++}$  constitutes the sole physiologically active calcium. However, in general, the ionization varies with the total  $[Ca]$ , the acidity, the weak acids, and especially with the proteins present. (See Figure 10.) It is impossible to state the serum  $[Ca^{++}]$  which will produce tetany, for apparently it is not only the absolute amount, but the degree of reduction from a previous level, and the rapidity with which this change is brought about, which produces tetany.<sup>12</sup> For instance, following operative removal of the parathyroid glands, tetany may supervene at serum concentrations of diffusible calcium which are ordinarily considered as above the tetanic level.

Recently there has been described a condition called puerperal paralysis in which the  $[Ca^{++}]$  in the serum is low. No tetany occurs; on the contrary, the animals are insensitive to stimuli. The  $[Mg^{++}]$  is increased.<sup>182</sup> A similar question is raised by the Meltzer phenomenon, in which magnesium narcosis is relieved by injection of calcium salts (see *Magnesium*). This paradoxical action shows that the effect of one anti-tetanic agent is counteracted by another, and remains unexplained.

General reviews of clinical and experimental tetany are given by Hess<sup>72</sup> and György.<sup>56</sup>

## Types of Tetany

The causes of the tetany syndrome can be divided into more or less arbitrary groups, characterized principally by the following conditions in the serum: (1) low [Ca]; (2) high [phosphate]; (3) high [ $\text{HCO}_3^-$ ]; (4) increased pH (alkalinity); (5) high [non-ionized Ca]; (6) toxic factors; (7) low [ $\text{Mg}^{++}$ ]. These have been summarized in Table 14.

Table 14.—Types of Tetany.

	Concentrations in the Blood Serum				
	Ca	$\text{Ca}^{++}$	$\text{PO}_4$	$\text{HCO}_3^-$	pH
1. Low calcium					
Ricketic or osteomalacic	low	low	high	normal	normal
Low calcium intake	low	low	high	normal	normal
Oxalate	low	low	normal?	normal	normal
Intestinal	low	low	?	normal	normal
Parathyroid	low	low	high	normal	normal
2. High phosphate	low	low	high	normal	normal or alkaline
3. High bicarbonate	normal	?	?	high	alkaline
4. Hyperventilation	normal	?	normal	normal	alkaline
5. Citrate	high	low	normal?	normal	normal
6. Toxic, guanidine	high	normal	normal?	normal?	normal?
7. Low magnesium	normal	normal	normal	normal	normal

*Low calcium.*—Tetany often occurs as a complication of rickets or osteomalacia. Ricketic children and rats usually have a high serum [Ca] and low [phosphate]. When treatment with vitamin D is inadequate or interrupted, tetany often supervenes. Latent tetany may be present when the serum [Ca] is about 8 mg./100 cc., and active tetany becomes manifest when the level reaches 4.5-6 mg. Calculated by the McLean and Hastings nomogram the [ $\text{Ca}^{++}$ ] is below 4 mg.<sup>119a</sup>

The equilibrium in the serum is peculiarly unstable, so that small amounts of ingested phosphate, which are without effect on normal infants or animals, lower serum [Ca] and cause tetany. Fasting of ricketic animals produces sufficient phosphorus from tissue metabolism to initiate tetany. Insufficient or interrupted therapy apparently improves the phosphate retention sufficiently to be the common inciting cause of tetany in ricketic infants.<sup>151</sup> The relation of low vitamin D to this condition has been described (p. 151).

If the calcium in the diet is very low, the phosphorus moderately high, and the vitamin D content deficient, rats develop rickets with low serum [Ca] and tetany ensues.<sup>18, 181, 184</sup> Puppies fed Mellanby's ricketogenic diet in which the calcium is not very low, also have low serum [Ca] and tetany. Premature infants who develop rickets often have a low serum [Ca] and tetany.

Oxalate, as would be expected from the low solubility product con-

stant of calcium oxalate, markedly diminishes the  $[Ca^{++}]$ , and is a potent producer of tetany.<sup>53</sup>

Celiac disease and sprue, intestinal diseases in which fat and calcium absorption are defective, lead to diminished calcium absorption, and are associated with rickets and low-calcium tetany.

Although high [phosphate] in the plasma has been emphasized in this connection it is not a necessary concomitant of low-calcium tetany. Infantile tetany especially may occur with a variable serum phosphate of 3-8 mg./100 cc.

Hypoparathyroid tetany and that which follows parathyroidectomy, which also fall in the low-calcium group, are discussed in Chapter 4 (pp. 110 and 112).

*High phosphate.*—Low calcium-high phosphorus intakes, whether by mouth or parenterally, produce tetany in normal, ricketic, or parathyroidectomized animals. It has been repeatedly emphasized that  $[Ca]$  and [phosphate] in the serum usually have an inverse relationship. Binger<sup>9</sup> was able to show (in dogs), not only the relation of phosphate injections to  $[Ca]$  in the production of tetany, but also the pH effect. Intravenous phosphate always depressed  $[Ca]$  in the serum, but only alkaline solutions produced tetany. Clinically in kidney disease there may be a retention of phosphate and a diminution of  $[Ca]$  in the blood serum without resulting tetany. This is due to the acidosis present. When large amounts of phosphates were given orally to dogs, tetany resulted from the acid, neutral or alkaline salts.<sup>158</sup>

Serum [phosphate] is not always increased in tetany; an increase may occur without diminished  $[Ca]$ . The [phosphate] in itself apparently does not cause tetany, but only insofar as it affects the  $[Ca]$ , and perhaps the  $[Mg]$ .

*High bicarbonate* concentration in the serum results either from loss of  $Cl^-$  or ingestion of  $HCO_3^-$ .

Gastric tetany is caused primarily by the loss of  $Cl^-$  by vomiting.<sup>70, 113</sup> In this case the  $[HCO_3^-]$  of the plasma sometimes increases to double its normal value. Definite increase in the pH may, but does not necessarily, occur. The  $[Ca]$  and  $[Ca^{++}]$  are little affected.

With large bicarbonate intakes, in the presence of damaged kidneys, when the usual efficient mechanism for removal of mineral anions is impaired, alkalosis and tetany may result.<sup>81</sup> In these cases, although the  $[Ca^{++}]$  has not been convincingly proved to be diminished, both the increased alkalinity and the high  $[HCO_3^-]$ , according to chemical equations, should be so operative.

*Alkalinity.*—Tetany may easily be brought on by forced breathing.<sup>26, 43</sup> This lowers the alveolar  $CO_2$  tension without alteration of the  $[HCO_3^-]$  of the plasma, and thus (according to the Henderson-Hasselbalch equation) the ratio of  $[HCO_3^-]$  to  $[CO_2]$  is increased, the

pH becomes higher, and tetany results. To compensate for the alkalosis, the  $[\text{HCO}_3^-]$  of the serum is lowered by excretion of an alkaline urine, the tetany is relieved, and normal equilibrium is restored.

Encephalitis may produce certain lesions in the brain which cause hyperirritability of the respiratory center, and hyperventilation. Tetany may ensue.

In the above cases hyperventilation caused alkalosis. Ordinarily this alkalizing effect of hyperventilation occurs only to compensate for an acidosis already present.

Measures which counteract alkalosis diminish or abolish tetany.

*High non-ionized calcium.*—Citrate injections have been shown to result in tetany.<sup>171</sup> Under such conditions the total serum  $[\text{Ca}]$  is high, but citrate combines with  $\text{Ca}^{++}$  to form a complex ion, and hence diminishes the  $[\text{Ca}^{++}]$ . This demonstrates in a convincing manner that it is the lowering of the  $[\text{Ca}^{++}]$ , and not of the total  $[\text{Ca}]$ , which causes tetany.

*Toxic factors.*—Guanidine tetany is included in the list because, so far as is known, it represents a different mechanism. Increased guanidine in the blood is usually found as a result of liver injury. Injected in sufficient amount it will cause tetany.

The finding of guanidine in parathyroid tetany led to the suggestion that it is the causative factor. Later studies indicate that it plays no important part either in the pathogenesis of clinical tetany, or in the factors which affect the ionization and physiological activity of calcium.<sup>27</sup> It may be regarded as one of a group of toxic agents which affect irritability. Strychnine is another such substance.

*Low-magnesium tetany* is given a separate grouping to emphasize that calcium ionization and pH are not the only mineral factors which influence the neuromuscular excitability. It is discussed under *Magnesium*.

It is obvious that these factors do not operate independently. Any combination of causes produces an intensification. Thus alkalosis increases the effect of phosphate administration, and vomiting intensifies that of hyperventilation. In the absence of vitamin D the phosphate effect is so marked that tetany occurs after fasting ricketic animals in spite of the concomitant acidosis. No condition in which all these factors operate at the same time has been described.

The opposite of the conditions which produce tetany lead to its amelioration. Thus high calcium intake, orally or parenterally, low phosphorus intake, acid-producing measures, fat-free diets, parathyroid extract, A. T. 10 and vitamin D, all inhibit the symptoms.<sup>164</sup> The interrelations of these actions, especially with parathyroid hormone, have been discussed in Chapter 4, *Internal Secretions*.

One further aspect of convulsive states related to mineral metabolism is found in the newer studies of epilepsy. This complex disease, or

group of diseases, has been found to be associated with edema of the brain, and anoxemia. That  $[Ca^{++}]$  may play a part has been suggested. The  $[Ca]$ , pH and  $[HCO_3^-]$  of the blood are within normal limits. Yet hyperventilation brings on seizures in epileptics more easily than it causes tetany in normal individuals. Alkalis intensify the symptoms, but starvation, acid therapy and diets which cause acetone body formation bring relief, especially in the young.<sup>105</sup> These measures suggest that the anion-cation relation, and hence the  $[Ca^{++}]$ , may be involved. Such treatments result also in dehydration. The beneficial effect of dehydration by water restriction demonstrates further relation of irritability to water metabolism (see Chapter 12).

## MAGNESIUM

### Distribution in the Body

The prominence of magnesium structurally has long been known, but its significance forms a comparatively new and incomplete chapter in physiology. It occurs in all the cells and fluids of the body in comparatively large amounts. The chief deposits are in the bones and muscles. Magnesium is closely allied to calcium, though it cannot replace it. In the muscles it is present in larger amount than calcium, but its function here is unknown. It has been pointed out that magnesium acts as a coenzyme in many of the enzyme systems concerned with phosphorus metabolism.

**Bone.**—Magnesium forms an integral part of the complex bone salts. The bone ash of different species varies in magnesium content only between 0.5 and 0.7 per cent.<sup>131</sup>

In a careful study of the bones of rats Hammett<sup>62, 63</sup> has shown that the magnesium decreases as the calcium increases in the bones, with slight but definite sex differentiation. Under stress magnesium can and does replace calcium in bone structure, to some extent. Conversely, both *in vivo* and *in vitro*, excess magnesium hinders calcification. The removal of the parathyroid glands, or of the thyroid and parathyroids, causes an increase in the magnesium content of the ash, and a decrease of calcium and phosphorus, although decalcification does not occur. In addition to diets low in vitamin A and high in calcium and phosphorus, those high in magnesium tend to produce urinary calculi. The interrelationships of the calcium, phosphorus and magnesium are complex, for it has been shown that addition of calcium to a high magnesium-low phosphorus diet prevents stone formation.<sup>114, 201</sup>

**Blood.**—Unlike calcium, the  $[Mg]$  of the red cells, 5.4-7.8 mg./100 cc., is greater than that of the serum, 1-3 mg./100 cc. Eighty per cent of the magnesium in serum is ultrafiltrable. This is a larger proportion of the total than is the diffusible calcium, and presumably is accounted

for by the small amount occurring as proteinate. Alterations in the protein, therefore, are not reflected in the [Mg] of the serum.

Little is known regarding the state of magnesium in the blood. It has been reported that it forms colloidal complexes with phosphate, but this has not been substantiated.

The serum [Mg] has not been shown to vary widely under ordinary conditions, nor is there any consistent alteration in pathological conditions. The [Mg] in serum may be low during menstruation, and is probably low during late pregnancy. It has been reported low in rickets, and in grass tetany of cows, although in infantile tetany values above normal are found.<sup>99, 153</sup>

The laws which govern the variations of [Mg] in the blood are not well understood, for although magnesium resembles calcium chemically, it is not equally affected by phosphates, protein, thyroxin, parathyroid hormone or vitamin D. Injections of calcium salts produce a rise in plasma [Mg], the peak coming about two hours after the injection. Phosphate injections not sufficient to cause a change in [Ca] result in a decrease in [Mg]. Injections of parathyroid extract cause a rise in serum [Mg], which disappears before the [Ca] increase thus induced reaches its maximum.<sup>163</sup> Vitamin D has also been reported to raise the [Mg] in serum only slightly. It has been reported in oxalate poisoning, which causes depression of the serum [Ca] and tetany, that the serum [Mg] becomes elevated.<sup>86</sup> These effects therefore clearly demonstrate not only that these potent preparations have small effect on [Mg], but also that magnesium and calcium operate very differently in the body.

The [Mg] of the cerebrospinal fluid is usually somewhat higher than that of the serum, and the ultrafiltrable fraction lower. Therefore, as far as the magnesium is concerned, the fluid cannot represent a true ultrafiltrate of the serum.<sup>202</sup>

### Effects of Magnesium Injection

**Magnesium narcosis.**—The earlier work of Ringer and Loeb and others with isolated muscles and tissues showed that magnesium is related to muscle irritability. Meltzer and Auer<sup>126</sup> demonstrated this effect dramatically in the intact animal. By parenteral injection of magnesium salts up to 0.1-0.2 gm./kg. they produced a profound narcosis and anesthesia. Larger amounts caused death. It was subsequently shown that this condition may be related to the [Mg] in the serum. A mild sedative or hypnotic effect is obtained when the serum level reaches 5 mg./100 cc. At about 18-21 mg./100 cc. profound coma is produced.<sup>138</sup>

When calcium salts were injected during this anesthesia, twice the fatal dose could be given without injury.<sup>126</sup> Further, if calcium was given to an anesthetized animal in the form of any soluble calcium salt,

it caused a temporary awakening, followed by a lapse into the original state. A second or third injection was necessary to overcome the condition.

Anesthesia can be induced by magnesium injection in man as well as in animals, and counteracted by calcium injection. Magnesium injection has been used subcutaneously, intravenously and intraspinally to overcome convulsions.

**Other effects.**—Injected magnesium salts are more effective than calcium or other salts in causing increase in blood sugar and glycosuria; 25-100 mg. of magnesium salt will raise the blood sugar to as much as 30 per cent above normal.

It has been reported that magnesium lowers the temperature in fevers.<sup>191</sup> The effect was found to be proportional to the rise in serum [Mg] thus produced rather than to the amount given. Each 2 mg./100 cc. increase in serum [Mg] was accompanied by a 1 °F. decrease in temperature.

Intravenous injection of  $MgSO_4$  has been shown to be of benefit in acute hemorrhagic nephritis of children.<sup>10</sup> It causes general improvement, diuresis, reduction of blood pressure and cessation of convulsions. Similar lowering of blood pressure in uremia has been reported.

### Intake

*Low-magnesium tetany.*—The necessity for magnesium in the diet has been amply proved by McCollum and his collaborators. Experiments on rats have shown that a magnesium intake of 0.18-0.30 mg./100 gm. of diet is too low to support life.<sup>100, 101, 104</sup> On such a regimen rats (and dogs) show vasodilation and convulsions ending in death. The blood plasma [Mg] is reduced to one-tenth its normal value, while the [Ca] remains normal. The electrical reactions demonstrate that this must be called low-magnesium tetany. Hence, magnesium as well as calcium must be considered in the pathogenesis of tetany.

The bones of these animals were unusually heavy, and contained an excess of calcium and phosphate. Magnesium was laid down in the bones during the experiment in spite of the small amounts in the diet, and reached values half those of the normal controls.<sup>142</sup>

Further studies have extended these findings to show that the [Mg] in the corpuscles as well as in the serum is greatly diminished.<sup>196</sup> The analysis of the soft tissue shows a slight diminution of magnesium. A great increase in calcium is found in the kidney.<sup>47</sup> This leads to a condition similar to nephrosis. The total amount of the body calcium is diminished.<sup>196</sup>

High calcium content of the diet intensifies the effects of magnesium deficiency, and raises the amount necessary to meet the minimum requirements.

Although no cases of low-magnesium tetany have been reported in man, there are two conditions in which it occurs in cattle. Grass tetany reported by Sjollema<sup>182</sup> and by Sjollema and Seekles<sup>183</sup> is a condition in which [Ca] in the serum is reduced to 6-7 mg./100 cc., and the [Mg] to 0.5 mg./100 cc. The animals are nervous, restless, suffer from loss of appetite, and finally go into convulsions and coma. The other form has been reported by Duncan, Huffman and Robinson.<sup>32</sup> When calves are fed whole milk, or whole milk supplemented with cod-liver oil, iron, copper and manganese, they show consistently low [Mg] in the serum. They lose their appetite, have convulsions and die. In both of these conditions the magnesium intake is adequate, and the cause of the condition has not been explained.

**High intake.**—In experimental animals high magnesium intakes are without harmful effects until amounts as large as 4 gm. of  $MgCl_2$ /100 gm. of diet are given. Then diarrhea, loss of appetite and death ensue. High intakes of magnesium cause only slight changes in the content of the serum or of the body.<sup>30, 120</sup>

The effect of high magnesium intake is shown less in the retention of magnesium than of calcium and phosphorus. High magnesium-low calcium and phosphorus diets produce rickets in young growing animals. This illustrates that it is not only the relation of high calcium-low phosphorus which prevents bone formation, but that any of the heavy metals which form insoluble phosphates can prevent adequate simultaneous assimilation of calcium and phosphorus (see p. 150).

### Absorption and Excretion

Tibbetts and Aub have recently made the most complete and satisfactory study of magnesium metabolism available.<sup>184</sup> Their conclusions have been used as authority for metabolic reactions of magnesium.

The magnesium in chlorophyll is in organic combination, and is probably liberated both in the stomach and in intestinal digestion. In the main its absorption and excretion parallel that of calcium. Like calcium, it forms insoluble soaps with fatty acids. These are emulsified and rendered absorbable in alkaline media by bile. A large proportion of the excreted magnesium is found in the feces. Most of this is unabsorbed material; the rest represents excretion into the intestine. It has been shown that, when injected intravenously, as much as 90 per cent is excreted in the urine.<sup>111</sup> Further, parenteral injections of magnesium carry an increased amount of calcium into the urine.<sup>29, 127</sup> Tibbetts and Aub found approximately 60 per cent of the excreted magnesium in the feces, under usual conditions, and with higher intakes an even greater proportion, 80 to 85 per cent. This is in substantial agreement with the data of Clark on normal individuals, and not essentially differ-



ent from the metabolism found in both breast-fed and artificially fed infants (see Tables 33, p. 325, 40, p. 336, 41, p. 337).

Acid ingestion causes a shift of magnesium from feces to urine, and increases the total excretion, as it does with calcium, but to a lesser extent. Similarly, when phosphate is added to the diet, the effect on the magnesium excretion is insignificant when compared to the effect on calcium. The striking feature of magnesium metabolism, which can be observed only when long fore and after periods are studied, is that unusually large excretion of magnesium may be continued for 3-10 days after administration is discontinued.

The magnesium excretion shows no marked alteration either in hyperparathyroidism, exophthalmic goiter or fatty diarrhea, which have special effects on calcium metabolism, or in Addison's disease, which has a close relation to sodium and chloride metabolism.

When magnesium lactate is given by mouth it increases the urinary calcium without diminishing the fecal calcium, and therefore causes increase in negative calcium balance, under conditions in which the calcium intake is low.<sup>22</sup> The excess magnesium shifts an increased proportion of phosphate to the feces, but does not cause a negative balance. When magnesium lactate is given with  $\text{NH}_4\text{Cl}$ , which in itself increases the calcium in the urine, the urinary calcium is further markedly increased, the fecal calcium is unaltered, and hence negative calcium balance is greatly increased. The phosphate again shows the same slight deflection to the feces, without change in its retention.

Phosphate additions are without marked effect on magnesium excretion.

High calcium intakes, which increase the calcium retention, are without effect on the proportions of calcium found in the urine and feces. Such calcium intakes do not diminish the magnesium balance, but cause a slightly larger percentage of the magnesium excreted to be deflected to the feces.

These facts can be correlated with the known distribution of magnesium and calcium in the body, for calcium is located predominantly in the bone, and magnesium is evenly distributed throughout the body. Thus magnesium is less readily mobilized by acid, which causes withdrawal of calcium from bone. It is also less closely associated with phosphate metabolism, for calcium and phosphorus necessarily act together in deposition or dissolution of bone.

*Catharsis.*—The poor absorption of magnesium has led to the use of its salts clinically as cathartics. Part of the effect is due to the lack of absorption, and part to their dehydrating action. The anion with which the magnesium is coupled is important. The absorption of the ions in the salt may take place to different degrees. The magnesium salts of strong acids, similarly to those of calcium, are acidogenic. They

thus increase the excretion of water by both bowel and kidney, and act not only as cathartics, but as diuretics. The salts containing non-mineral anions—carbonate and lactate—have much less cathartic action than the chloride and sulfate.

### Retention

Retention of magnesium is related to the immediate need of the body, as there seems to be no depot for magnesium storage comparable to that for calcium and phosphorus.

Infants may have large retentions, up to 30 per cent or more of the intake, because magnesium is required to maintain normal structure of the new tissue formed in growth. Balance studies show great irregularity and some negative balances. The same is true of children, regardless of whether the intake is high or low.<sup>199</sup>

The adult approximates equilibrium regardless of the intake. When the amount ingested is increased to 3 or 4 times the usual intake, the retentions vacillate between plus and minus.<sup>194a</sup> In pregnant and lactating women, even on high intakes, the retentions are also irregular. Clark's data (Table 39, p. 333) show that all five of his prison subjects were in negative balance over the whole of a 28-week period, although the intake was of the order of 0.25 gm. per day. They showed positive calcium balances, which may be attributed to their previous condition of calcium deficiency. Therefore they were probably excreting the extra magnesium which they had previously deposited in the bones in lieu of calcium. With magnesium intakes increased up to 0.75 gm./day, one individual continued in negative balance.

### Requirement

Very little is known about the requirement for magnesium, because it has been little studied, except incidentally in relation to the other positive minerals. The amount usually ingested varies with the type of food. The two main sources of magnesium in the average diet are milk and vegetables. Owing to its occurrence in chlorophyll, green diets are high in magnesium. The average intake of magnesium per 2500 Cal. was found by Tigerstedt,<sup>195</sup> in Finland, to be 0.74 gm. per day, and by Sherman,<sup>172</sup> in the United States, 0.27 gm. Wendt<sup>204</sup> offered as a standard, 0.01 gm./kg. of body weight, which amounts to 0.70 gm./man/day.

Tibbetts and Aub<sup>194a</sup> have shown that 0.22 gm./day gave small positive balances on a neutral diet, with either low or normal calcium intakes. The normal breast-fed or artificially fed (milk plus water in equal amounts) infant obtains about the equivalent of the Finnish standard. It must be concluded that the body can adapt itself to maintain

equilibrium on widely divergent levels of intake. No untoward effects are manifest whether these are low or high. The usual American intake seems to be adequate.<sup>20</sup>

It can be seen that our knowledge of magnesium metabolism is very fragmentary. Although magnesium is widely distributed not only in the body but in foodstuffs, its physiological significance is not well understood, and will undoubtedly be the subject of future development. Its importance with relation to irritability does not disclose its whole function in the body. Clinically it is used for antispasmodic and diuretic effects when given parenterally, and for cathartic, diuretic, alkalizing or acidifying effects when taken by mouth.

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## Chapter 7

# Phosphorus

### Compounds of Phosphorus

The chemistry of the compounds of phosphorus forms one of the most interesting and difficult branches of biochemistry and physiology. Phosphorus is distributed so widely as to be present in all the body cells and fluids, and occurs in all natural foodstuffs. The metabolism of phosphorus is related to that of the other minerals and to protein, fats and carbohydrates as well. The important body functions upon which advances in the chemistry and physiology of the compounds of phosphorus have shed most light are ossification, muscular contraction and acid excretion. A storehouse of information is still to be found in the monograph of Forbes and Keith.<sup>9</sup> Recent reviews are available.<sup>20, 41</sup> The metabolism of phosphorus is so closely related to that of calcium that they are necessarily discussed together in many phases of their body functions. Chapters 6 and 14 especially contain many references to phosphorus.

The proteins include the phosphoproteins, such as casein, and the nucleoproteins. The phosphatides or phospholipids include structural and circulating compounds such as the cephalins and lecithin. Knowledge is rapidly accumulating concerning the hexose phosphates, glycerophosphates, creatine phosphates, nucleotides and coenzymes. All these compounds contain phosphorus in the form of esters or of phosphoric acid. When they are built up or split in the body a frequent intermediary stage is that of orthophosphate. So far as is known the meta- and pyrophosphates found on analysis result from the preliminary steps in the analysis, and do not function as such in the body, and if given must be hydrolyzed before they can be utilized. The inorganic phosphates are distributed throughout the body fluids, in both the intercellular and intracellular fluids, and excretions and secretions.

Some of the compounds of phosphorus are enumerated under the headings of the systems in which they occur. Others, however, are so widely distributed that they are best mentioned in general discussion.

**Proteins.**—The *phosphoproteins* are discussed briefly under *Intake*, page 186.

*Nucleoproteins* occur in every cell and every nucleus, both animal and plant. The nucleic acid phosphorus constitutes about one-half of

the total phosphorus content of the organs which are rich in cells—the liver, pancreas, thymus and spleen. Nucleic acids contain phosphate ester linkages with purines, pyrimidines and carbohydrates. The chemistry of these compounds has been summarized by Jones<sup>18</sup> and by Levene,<sup>24, 25</sup> and need not concern us here. Little is known of their relation to minerals in life processes, but when metabolized they furnish purines in combination with phosphate, or carbohydrates in combination with phosphate. The recent identification of many compounds in muscle has further broadened our knowledge of these substances. In addition to adenylic acid (adenosine phosphate), adenosine diphosphate and adenosine triphosphate ("pyrophosphate") are vital to muscle metabolism.

The body contains a variety of enzymes which are capable of hydrolyzing nucleic acid or any of the smaller groups. The purine base which is split off is oxidized and excreted as uric acid (2, 6, 8-trioxypurine). The residue then consists of carbohydrate and phosphate, which may be still further acted upon by enzymes. On the other hand, phosphate may be hydrolyzed from mononucleotide leaving purine combined with carbohydrate. This reaction runs to equilibrium, and is reversible; it is also one of the fundamental reactions in muscle physiology (see p. 180).

**Flavin.**—Flavin, which has recently become identified with vitamin B<sub>2</sub>, has been shown to enter into combination with phosphate to form ribiflavin (lactoflavin-5-phosphoric acid). This in turn may combine with protein. Similarly, dimethyl- $\alpha$ -1-araboflavin-phosphoric acid combines with protein to form compounds with vitamin activity. The explanation of the action of these compounds, especially in relation to mineral metabolism, must await future developments.

In addition it is known that flavoprotein is of importance in oxidation-reduction systems. It has been shown to reduce both methylene blue and cytochrome-c. On the other hand flavoprotein also reacts with reduced pyridine compounds which have been shown by Warburg<sup>53</sup> to be important in fermentation, glycolysis and respiration.

**Phospholipids.**—The older term for these compounds, especially in Germany, is *phosphatide*, and the newer American term is *phospholipid*. The two may be used interchangeably. These compounds occur in great variety, and are essential for many of the body functions. The physiological activity of any tissue is related to the phospholipid content. The concept is fairly well established that the phospholipid content of any given tissue is a fixed characteristic of that tissue, and that it is maintained with great tenacity. However the amount is greater in most of the organs of the young than in those of the adult. In Schüller-Christian disease and Niemann-Pick's disease phospholipids accumulate in the brain and liver. In the latter condition the sphingomyelin is diminished

to half, and the monoaminophosphatides are increased to about three times the usual amount. The cholesterol metabolism is also distorted.

The phospholipids form colloidal solutions in water, which permits their transport in aqueous media and across cell membranes. Their unsaturated fatty acid radicals may serve as a reversible oxidation-reduction system in cells or at the surface of cells. Further, they are important in immunological reactions. The water content of cells has been related to the balance between the lecithin and cholesterol contents. The theory that phospholipids are responsible for the transport and resynthesis of milk fat in the mammary gland<sup>31</sup> has recently been questioned.<sup>12</sup>

These substances are not only intermediate between carbohydrates and fats, but may be essential in the metabolism of both. Moreover they form compounds with protein (ovovitellin). Compounds with glucosides and carbohydrates are reported; and jecorin (perhaps a mixture) which is obtained from liver, muscle and brain, contains lecithin, and also glucose and sulfur. For the present it is sufficient to consider only the three principal phospholipids.<sup>1, 37, 47</sup>

*Lecithin* is like the true fats except that the third molecule of fatty acid is replaced by the choline ester of phosphoric acid.

*Cephalin* is similar except that the choline is replaced by amino ethyl alcohol. When this is hydrolyzed, glycerophosphoric acid is obtained. It is very difficult to separate the cephalin and lecithin, but it has been accomplished by the methods of Maclean,<sup>28</sup> and also of Levene.<sup>24</sup> Lecithins are complex, because the fatty acid radicals are mixed and contain palmitic, stearic, oleic, linolic, linoleic and arachidonic acids. Most lecithins probably contain one saturated and one unsaturated fatty acid. Cobra venom acts to split off the unsaturated fatty acid group, to form lysolecithin or lysocephalin, which hemolyze red blood cells.

*Sphingomyelin*.—This substance occurs in large amount in the brain and the so-called "protagon" has been thought to be a mixture of this substance and cerebrosides. In sphingomyelin the glycerol of lecithin is replaced by sphingosine containing 17 or 18 carbon atoms.

**Carbohydrates.**—Study of the carbohydrate compounds with phosphate at present constitutes an extremely active front of scientific endeavor. The development has been so recent and so rapid that it is difficult for the non-expert in the field to orient himself, much less pass any critical judgment.

The formation from carbohydrate of alcohol, lactic acid and CO<sub>2</sub> which takes place in yeast fermentation, muscle activity and metabolism are all now known to be intimately related to phosphorylation (or PO<sub>4</sub> esterification). The compounds recognized to be important are the hexosemonophosphates, hexosediphosphates, phosphoglycerol, phosphoglyceric acid, phosphotriose, phosphopyruvic acid, phosphodihydroxyacetone, phosphoglyceraldehyde (and also the nucleotides containing pentose or

hexose, and creatine phosphate). These are discussed under *Muscle*, see below.

### Distribution of Phosphorus in the Body

The distribution of body phosphorus was originally calculated by Voit<sup>52</sup> and corroborated by Sherman.<sup>44</sup> About 80 per cent lies in the skeleton, 10 per cent in the muscles, and 1 per cent in the nervous tissue. The phosphorus content of the body and its parts has been discussed in Chapter 2, and therefore only special forms of phosphorus are here considered. These have been reviewed by Kay.<sup>20</sup>

Recently a radioactive phosphorus has been prepared by the bombardment of the isotope  $P_{32}$  with deuterons in the cyclotron. This promises great advances, as a new physiological method is thus made available. Such atoms may be detected and located by the electroscope. The distribution of phosphate in the body immediately following administration may be determined, and its subsequent fate followed. By this technique the sites of phospholipid deposition were determined to be the liver, intestine and kidney.<sup>39</sup> A study of ingested phosphate demonstrated that 30-40 per cent of the phosphate was found in the intestinal tract after 8 hours. Of the injected or absorbed phosphorus 20-30 per cent was excreted in the urine, and only 3 per cent by the intestine. The bone contained a larger fraction of the phosphorus than any other organ; the liver the next most, per gm. of fresh tissue; and the brain contained least of any of the tissues analyzed.<sup>4</sup>

**Bone.**—Phosphorus occurs in the skeleton not only in larger amount, but also in higher concentration than in any other part of the body. This phosphorus occurs almost exclusively as inorganic phosphate in the solid phase of the complex bone salts (see p. 31). Considerable phospholipid is present in bone marrow. Phosphatase is discussed under *Calcification* (p. 142).

**Muscle.**—In spite of the importance of the phosphorus in muscle, the actual distribution of the compounds and their phosphorus content is not known. For the lack of any adequate data on man, Eggleton<sup>5</sup> was forced to give the following as the composition of muscle:\*

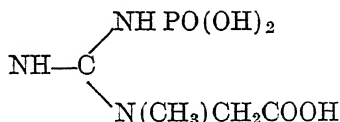
Approximate Composition of a Resting Skeletal Muscle of a Frog.

Substance	%	Substance	%
Water .....	80.	Creatine .....	0.08
Protein .....	17.	Adenosine triphosphoric acid	
Fat and lipids .....	0.2	(adenylpyrophosphate) ...	0.25
Glycogen .....	0.7	Urea .....	0.01
Hexose monophosphoric ester	0.05	Orthophosphate (as $H_2PO_4$ )..	0.045
Creatine phosphoric acid....	0.45	Chloride .....	0.05
Lactic acid .....	0.015	Bicarbonate .....	0.03
Carnosine .....	0.25		

\* Reproduced by permission of W. B. Saunders Co.

Eggleton's comment on this table is: "The figures do not add up to 100 per cent for we do not know all the substances present, and nobody has analyzed a single muscle for all the known substances. From outside evidence we know that some of the protein is of structural value. The voluntary muscles of other vertebrates give similar results, though there are some characteristic differences in quantitative relationships. Thus the muscles of active animals contain, on the whole, more creatine, free and combined, than those of sluggish animals, and even in one and the same animal the more rapid muscles contain more than the others. Again, some vertebrates have little or no carnosine in their muscles, but have instead methyl carnosine (anserine). The muscles of invertebrates, on the other hand, contain neither creatine nor carnosine, but they contain arginine, both free and in combination with phosphoric acid. Carnosine and anserine are peculiar to muscle tissue, and presumably have some part to play in the special function of muscle, but no good experimental evidence has been put forward which gives any suggestion as to what this function is."

*Creatine phosphate*.—Creatine (methylguanidineacetic acid) has long been known to occur in muscle and has been classed as an extractive. The important contribution by Fiske and SubbaRow<sup>8</sup> demonstrated that it occurred as creatine phosphate:



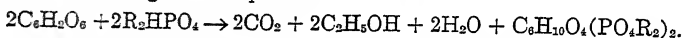
The fundamental action of this compound in muscle metabolism is the groundwork of all modern theories of muscle physiology. The second dissociation constant of the acid is  $2.6 \times 10^{-5}$ , and therefore at the pH of the cell it dissociates into anions carrying more electric charges than those of  $\text{H}_3\text{PO}_4$ . Hence when the compound is hydrolyzed, and phosphate is set free, because this is an anion of a weaker acid ( $\text{pK}_2 = 1.6 \times 10^{-7}$ ), fewer cations are needed to neutralize it. The cations thus freed may neutralize the lactic acid formed in metabolism and thus prevent an increase in acidity. The actual change in acidity will depend upon the initial pH and the amount of creatine phosphate hydrolyzed and the amount of lactic and other acids formed.

During muscular contraction the phosphocreatine is split and during the recovery phase it is resynthesized. Fiske and SubbaRow were able to show that the muscles of the cat contained about 75 mg. of creatine phosphate per 100 gm., and 25 mg. of inorganic phosphate. They demonstrated further that, when the muscle was completely fatigued, the creatine phosphate was entirely converted into inorganic phosphate.

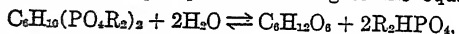
*Cozymase.*—Warburg<sup>54</sup> has identified a diphosphopyridine nucleotide as cozymase, which is distinguished from the coenzyme triphosphopyridine of Robison.<sup>41</sup> Cozymase has been identified with cytochrome-c, or yellow ferment.<sup>7, 49</sup> The relations between cozymase and the phosphocarbohydrates and phosphocreatine are discussed below.

*Chemistry of muscle contraction.*—The changes which the phosphorus compounds undergo in the body are exceedingly complex. As an example of the chain of events in metabolism the reactions during muscular contraction are briefly outlined here. In order to introduce the discussion of the mechanism of muscle contraction it is well to describe the analogous but simpler process of fermentation.

Carbohydrate esters of phosphate were discovered by Harden<sup>38</sup> in 1905, in the products of yeast fermentation. He was able to identify hexosediphosphate and two different hexosemonophosphates, only one of which reduced Fehling's solution. He showed that the addition of neutral phosphates increased the CO<sub>2</sub> formed when glucose was fermented by yeast. The reaction which was nearing completion was accelerated for a time by the addition of phosphate. Further addition caused a second increase in the rate of fermentation. Equivalent amounts of CO<sub>2</sub> and alcohol are produced according to the equation:



Thus one molecule of sugar forms one molecule of hexosediphosphate and the other breaks down to CO<sub>2</sub> and alcohol. No free phosphate remains. If there is a secondary breakdown of the hexosephosphate according to the equation,



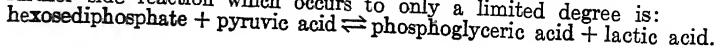
the original reaction may proceed further. These two reactions progress simultaneously.

In 1933 Embden<sup>6</sup> proposed the thesis that additional intermediary products are formed. This Meyerhof applied to both alcoholic and lactic acid fermentation. The hexosediphosphate is converted into triosephosphate which by an oxidation-reduction process yields α-phosphoglycerol plus 3-phosphoglyceric acid. Phosphoglyceric acid breaks down to pyruvic and phosphoric acids. Pyruvic acid is converted by yeast juice into CO<sub>2</sub> plus acetaldehyde. Acetaldehyde, glucose and phosphate then give rise to alcohol through the intermediary action of hexosediphosphate, and the ester is oxidized to phosphoglyceric acid. This is an amplification of the equation given above for the action of phosphate in alcoholic fermentation.

It was subsequently shown by Meyerhof<sup>32</sup> that the ester formed is phosphodihydroxyacetone. The enzyme zymohexase is catalyzed by Mg<sup>++</sup>. The probability is that phosphoglyceraldehyde is converted into the above ester. Meyerhof showed that the conversion of (−3)phosphoglyceric acid into pyruvic and phosphoric acids takes place in three stages with the formation of two new phosphoric esters, (+)2-phosphoglyceric acid and phospho-enol-pyruvic acid. It has further been discovered that phosphate is easily transferred from phosphoglyceric acid to glucose. No coenzyme is required for these reactions, but it is essential for the third stage, the formation of pyruvic acid, which is irreversible. The coenzyme is adenosinetriphosphate, which is hydrolyzed to furnish the necessary phosphoric acid groups, and is later resynthesized.

By poisoning with iodoacetic acid, it has been shown that phosphopyruvic acid rapidly forms hexosediphosphate, acetaldehyde and CO<sub>2</sub>, which are essential to the progress of the fermentation of alcohol. The term hexosediphosphate in this case really represents a complex mixture of phosphate esters.

A further side reaction which occurs to only a limited degree is:



The reactions which take place during the contraction of muscle have recently been summarized.<sup>32, 33, 38</sup> It has seemed desirable to present neither the many conflicting viewpoints nor a mass of experimental data. In the main, therefore, we have followed the line of argument developed by Meyerhof.<sup>32</sup> In general the reactions are similar to those of yeast fermentation except that lactic acid is produced instead of alcohol.

The earlier brilliant researches of Meyerhof and Hill showed that approximately four-fifths of the glycogen is transformed into lactic acid which is resynthesized to carbohydrate, and only one-fifth is burned. These reactions in muscle are further related to the other reacting systems present.

It seems probable that the hexosephosphate in resting muscle is present largely as hexosemonophosphate. Before it can be utilized it must be converted into hexosediphosphate; it then undergoes a series of degradations similar to those which have been described above.

Phosphocreatine is split to form creatine and phosphate when muscle contracts. The inorganic phosphate of blood is increased after exercise.<sup>11</sup> This phosphate is required for the phosphorylation of the carbohydrate. It is also necessary that the phosphocreatine be resynthesized during recovery. Even during fatigue there is a balance between hydrolysis and resynthesis. This resynthesis can take place only through the intermediation of a second system, the adenosine phosphate. The breakdown of creatine phosphate causes adenosinemonophosphate and adenosinediphosphate to form adenosinetriphosphate. Conversely the breakdown of adenosinetriphosphate causes the resynthesis of creatine phosphate. The adenosinetriphosphate necessary for the above reaction is derived from the interaction between adenosinephosphate and phosphopyruvic acid in which the latter breaks down to lactic acid and gives its phosphate to the adenosine. This reaction was discovered by Parnas.<sup>38</sup>

This furnishes only one half of the phosphate necessary for the reversion to creatine phosphate. The breakdown of phosphopyruvic acid is the only source of phosphate given off from an intermediary product of carbohydrate breakdown. The other half is supplied by the coenzyme system of Warburg. Exactly as the adenylic system acts as a catalyzer of phosphate transfer, so cozymase acts as a catalyzer of hydrogen transfer (neither appears in the final reaction). The hydrogen is transferred by the oxidation of triosephosphoric acid to phosphoglyceric acid; the hydrogen thus carried is split off when pyruvic acid is converted to lactic acid. At the same time creatine and phosphate are converted to creatine phosphate. The energy from the oxidation-reduction is used for the resynthesis.

That the reactions as given are valid is determined not only from the isolation of these compounds from tissue but also by subsequent study *in vitro*—the compounds isolated might have been formed after the



death of the tissue or during their removal from the tissue. Further evidence is offered by the discovery of enzymes which catalyze these reactions. The energetics of these compounds *in vitro* have been shown to correspond to the heat transfers and alterations in acidity which actually have been observed, not only in degree, but also with regard to the time relations of events in living muscle.

In addition to the above reactions several new lines of investigation have been developed. It has been shown that glutathione is the coenzyme which converts methyl glyoxal to lactic acid. This reaction is inactivated by iodoacetic acid. This is a second mechanism for the formation of lactic acid.

In the absence of vitamin B<sub>1</sub>, pyruvic acid is known to accumulate in the blood. The possibility that this too may be involved in the metabolism of carbohydrates is therefore obvious.

Recently it has been claimed by Verzár<sup>51</sup> that the action of adrenal cortex on phosphorylation of fats and carbohydrates is necessary for the formation of lactoflavin phosphate and yellow ferment.

Many or all of these transformations which are either reversible or irreversible oxidation-reductions are catalyzed by specific and nonspecific enzymes and coenzymes, organized as hydrogen carriers or electron carriers, and by inorganic magnesium and manganese. Therefore one gets almost as complete a story from the description of the action of the known enzymes as from the known chemical reactions and products.<sup>26</sup>

The explanations of the process as developed for the fermentation of sugar, the contraction and metabolism of muscle can be described only as the simultaneous integration of a number of chemical reactions. The story is so brilliant and satisfying that it leaves little doubt as to the nature of the mechanisms involved. However the rapidity of the development, the many new compounds involved and the intricacy of the pattern which they weave leaves one breathless. A perspective is at present impossible, and the possibility of new mechanisms and new compounds being discovered is so great that further details do not seem justified in a review of this type.

**Brain.**—Although since 1719 phosphorus has been known to occur in the brain, the researches of Thudichum,<sup>50</sup> published in 1884, laid the groundwork for our present knowledge. He analyzed over a thousand brains. In the mind of the laity the importance of phosphorus in the brain is firmly set, as is evidenced by such statements as that fish contain phosphorus, and are therefore "brain food," or the oft-quoted German aphorism, "Ohne Phosphor keine Gedanke."

Chemically speaking the phosphorus is prominent, and constitutes 0.36 per cent of the fresh brain, or 1.45 per cent of the dry matter.<sup>22, 23</sup> This is about one-fifth the amount of nitrogen, and 3 times that of sulfur.

It was shown by Bibra in 1853 that phosphorus was located in the fat and was a constituent part of it. This we now know to be the phospholipids lecithin, cephalin and sphingomyelin. A smaller fraction is found in the nucleoproteins, which constitute about one-half of the brain proteins. The proteins comprise one-third of the dry matter, the phosphatides one-fourth and the cerebrosides one-eighth.

The distribution of phosphorus in the brain is given in Table 15. It is striking how large a portion of the phosphorus is lipoidal and how small is that in the protein. The greater portion of the lipid phosphorus (and

Table 15.—Distribution of Phosphorus in the Brain.\*

	6 Weeks	% Total Phosphorus					
		2 Years		Whole	19 Years		Whole
		Gray	White		Gray	White	
Protein P .....	5	6	6	6	5	5	5
Lipid P .....	54	62	72	67	63	81	72
Water-soluble P....	41	32	22	27	32	15	23

\* Koch and Mann.<sup>22</sup>

sulfur) is found in the white matter. It is also clear that the extractive phosphorus or water-soluble phosphorus forms one-fourth to one-third or more of the total. All this phosphorus is certainly not inorganic. The nature of this fraction is not yet clear.

Studies of the metabolism of the brain have only recently been undertaken.<sup>37</sup> The evidence discloses that the mechanisms of carbohydrate breakdown which depend upon the intervention of phosphates are similar to those already discussed.

**Blood.**—The analytical data in regard to the distribution of phosphorus compounds is most complete for the blood, because this tissue is so readily available. The present values may be subject to revision as the methods of analysis are improved. The difference between the total phosphorus and that soluble in acid is called phosphatide or phospholipid phosphorus. This fraction may be obtained also by extraction with alcohol and ether. The inorganic phosphate is determined by analysis. This value is subtracted from that of acid-soluble phosphorus and the remainder is called ester phosphorus. This residue contains not only those esters which can be split by enzymes, but also those which are not so split, and in addition nucleic acids, phosphocreatine and the rest of the phosphorus compounds known and unknown.

The phosphorus compounds are distributed quite unequally between the cells and plasma. According to the information desired, the significant data must be sought in the study of the whole blood, or cells or plasma. Ordinarily the cell volume comprises 45 per cent of the blood, but this varies considerably both with age and pathological conditions, especially anemia.

The amounts and distribution of the phosphorus fractions are given in Table 16. These average values have been compiled from the litera-

Table 16.—Phosphorus Partition in the Blood of Adults.\*

	Total P		Lipid P		Ester P		Inorganic P	
	(mg./ 100 cc.)	(mM/l.)	(mg./ 100 cc.)	(mM/l.)	(mg./ 100 cc.)	(mM/l.)	(mg./ 100 cc.)	(mM/l.)
Whole blood ...	38	11.9	12	3.9	23	7.4	3	1.0
Cells .....	68	21.3	17	5.5	50	16.1	?	
Serum .....	13	4.2	8	2.6	1	0.3	3-4	1-3

\* Compiled from the literature.

ture, in preference to the wide fluctuations which have been given by Peters and Van Slyke.<sup>40</sup> Considerable weight has been given to the values of Kay and Byrom<sup>21</sup> for whole blood.

*Ester phosphorus.*—These authors have found that of the ester phosphorus only 6.2-6.9 mg./100 cc., or 28 per cent, is hydrolyzable by a phosphatase obtained from the bones of young rats. The blood also contains various enzymes which are capable of splitting (or synthesizing) these compounds. The one that has been most studied by Robison and Kay is that which splits hexosephosphate. The importance of this enzyme, called phosphatase or phosphoesterase, has been considered under *Calcification* and *Rickets*. The question of whether the phosphates can be split within the cells, transferred to the serum, and then to the site of calcification has not yet been answered. Kay<sup>19</sup> and Haldane<sup>15</sup> found that  $\text{NH}_4\text{Cl}$ -acidosis caused a reduction of the acid-soluble phosphorus to about one-third of its normal value.

The acid-soluble phosphorus in the blood cells is now being studied intensively. The main constituent found is phosphoglyceric acid.<sup>14, 55</sup> Triphosphate (sometimes called pyrophosphate) is present. It is probable that the compounds may be found to be as complicated in their interactions as those found in muscle.

*Inorganic phosphate.*—The value given for whole blood is perhaps subject to revision, because some of the phosphorus in the cells may have been hydrolyzed in the process of analysis. The value given for whole blood in Table 16, assuming that the inorganic phosphate is all in the serum, equals 5-6 mg./100 cc. of serum, a value known to be too high. Buell<sup>3</sup> found that when special precautions were taken in handling the blood, the cells contained no inorganic phosphate. When blood stands at room temperature for several hours, and the plasma is then separated, the value is found to be two or three times as high as when it is separated immediately. This phosphate could have come only from splitting of products within the red cells. However, no increase in phosphate in the plasma is found following muscular exercise.<sup>11</sup> Hence, the split products must be bound in non-diffusible compounds, or the muscle cell is not

permeable to inorganic phosphate, or the phosphate must be removed from the plasma as fast as it is introduced.

*The state of the inorganic phosphate* in the serum has been extensively studied and reviewed by Schmidt and Greenberg.<sup>43</sup> The main question is whether the phosphate is combined with calcium or other positive minerals to form non-ionized or colloidal salts. At present the best evidence indicates that the inorganic phosphate should, under normal conditions, be regarded as completely ionized.

*Lipid phosphorus* can be calculated in terms of lecithin by multiplying by the factor of 25, and therefore represents 300 mg./100 cc. of whole blood. Usually the phospholipids, cholesterol and neutral fat in the blood increase or decrease proportionately.

*Nucleotide phosphorus*.—Jackson<sup>17</sup> found that the nucleotides represent about 2 mg. of phosphorus per 100 cc. of human blood.

*Changes during life*.—Infants have a serum inorganic phosphorus concentration of 5.0-6.6 mg./100 cc. This value gradually diminishes throughout childhood until it reaches 3-4 mg. in normal adults. The ester phosphorus concentration in the cells is 80 mg./100 cc. of cells at birth, rises to 90 at two years, and then falls gradually to the adult level of 50.<sup>48</sup> This value is obscured in the analysis of the whole blood, for in infancy the volume of the corpuscles is at a minimum. Because the phosphorus in the cells is about five times that in the serum, the total phosphorus values in whole blood increase with the increasing proportion of cells to serum.

*Cerebrospinal fluid* contains about half the concentration of phosphate found in the blood plasma. No organic fractions have been determined with certainty.

*Effect of intake*.—Variations in the serum phosphate concentration are of great interest because they are directly related to the other minerals. Ordinarily there is an inverse relation between the blood serum [Ca] and [phosphate]. When the serum [Ca] is low the [phosphate] is high, and conversely when the [phosphate] is high the [Ca] is low. But this inverse relation is not always present. Both may rise or fall together. Administration of large amounts of calcium salts by mouth causes an increase in the serum [Ca] and usually, but not invariably, a small rise in the serum [phosphate]. The rise takes place in about two hours, and the [phosphate] returns to the initial level in four hours.<sup>13</sup> In clinical rickets, tetany may supervene whether the [phosphate] is high or low.

As is reported under *Rickets* (p. 149), a low phosphorus diet, especially when combined with high calcium intake, in the absence of vitamin D, causes a low serum [phosphate]. This is the condition ordinarily found in rickets. But the reverse condition, namely, high phosphorus-low calcium intake, or low phosphorus-low calcium intake, each with

corresponding serum values, also may produce rickets. As the intakes of both rise at a given ratio of Ca/P, the serum concentration of each increases. Thus the blood reflects both the proportion of calcium to phosphorus and the absolute amounts of each in the diet.

Of the vitamins only vitamin D has an effect on the serum inorganic phosphate concentration; this subject has been considered under *Calcification* and *Rickets*. A discussion of the effects of parathyroid hormone will be found there and also under *Tetany* and *Parathyroid Glands*. Adrenalin causes a lowering of the blood serum [phosphate] followed by an increase. These changes are often irregular. The adrenal cortical hormone has been studied in relation to the action of vitamin B, and apparently both form links in the chain of reactions necessary for the phosphorylation of fats and carbohydrates. Administration of posterior hypophysis causes a transient increase in blood serum [phosphate]. Thyroxin causes an increase in serum [phosphate]; phosphate is supposed to reinforce the action of thyroxin.

*Relation to carbohydrate metabolism.*—Removal from the blood and subsequent metabolism of carbohydrate requires both insulin and phosphate. In normal individuals after the sugar in the blood has been increased by the ingestion or injection of carbohydrate, a diminution in the plasma [phosphate] is observed.<sup>2</sup> Conversely, an increase in the [phosphate] causes lowering of the blood sugar. In severe diabetes no decrease in phosphate is observed after ingestion of sugar. When insulin is administered to diabetics and carbohydrates metabolized, there is a diminution in the serum [phosphate]. Further, the action of insulin in lowering blood sugar is enhanced by phosphate; in other words, the sugar and phosphate are removed from the blood in conjunction with each other. Insulin causes an augmentation of organic phosphorus in the blood.

### Metabolism

**Intake.**—It is obvious from the prominence of phosphorus in the composition of the body, to the last cell, that phosphorus forms an essential part of the diet. Osborne and Mendel<sup>36</sup> showed that moderate restriction of phosphorus intake constitutes a limiting factor in growth. The normal, optimal and minimal intake, the paths of excretion and balances of phosphorus are considered in Chapter 14. Some of the more specific problems dealing with phosphorus alone, such as the forms of phosphorus in intake and excreta, will be mentioned here.

Unlike sulfur, organic forms of phosphorus are not essential for maintenance of life. McCollum *et al.*<sup>30</sup> showed that a hen could produce more organic phosphorus in the eggs she laid than that originally contained in her whole body plus that consumed in the food. It is evident that the body can synthesize nucleic acids, for young mammals develop on milk

only. Further, embryos develop from eggs, and the salmon converts its muscles into eggs and sperm. This development may take place without purines being furnished.

However, probably not all forms of phosphorus are equally well utilized. The availability depends upon both the type of phosphorus compound and the other constituents of the diet. The studies of Forbes concerning the superiority of organic phosphates such as glycerophosphates, are difficult to appraise, for the total consumption of the different experimental groups was not the same.

The forms of phosphorus ingested vary widely with the type of food eaten. Insofar as animal food is consumed its various compounds have been mentioned in this and other chapters. There are in addition a few foods which contain compounds of especial interest.

*Casein* occurs as one of the two principal proteins of milk of all species, the other being albumin. The ratio of casein to albumin as well as the absolute amounts of each differ in various species. The phosphorus content of casein also varies with species; it has been reported as 0.8 per cent in cow's milk, 0.24 per cent in human milk, and 1.04 per cent in ass's milk. Attempts have been made to show that the phosphorus in milk is similar to that of nucleic acids, but Osborne has stated that practically nothing is known as to the nature of this phosphorus, not even whether it is present as  $\text{PO}_4$  or as an organic complex.<sup>34</sup> Like other proteins, casein has the capacity to form caseinates, especially calcium caseinate.

The most characteristic property of casein is its capacity to be coagulated by rennet. Probably rennet brings about a transformation into paracasein and the lime salts cause the precipitation.

So far as is known the metabolism of casein does not differ from that of other proteins because of its phosphorus content. It is synthesized by the mammary gland.

*Ovovitellin* is a combination of lecithin and albumin found in the yolk of hen's eggs. According to Osborne,<sup>35</sup> the lecithin component varies from 15 to 30 per cent. Some have reported that preparations of paraneuclins derived from the enzymatic digestion of egg yolk contain from five to ten per cent of phosphorus. There is some question as to whether phosphorus is associated with the proteins as an impurity.

*Phytin*.—Inositol-phosphoric acid, or phytin, is probably a compound of six phosphoric acid groups with inositol, which is hexahydroxycyclohexane,  $\text{C}_6\text{H}_6(\text{OH})_6$  (not a carbohydrate). Phytin occurs only in plants. There is some question as to whether it is readily utilizable by man.

The phytin phosphorus represents 30-70 per cent of the phosphorus of both corn and gluten. The standard diet for the production of rickets in rats comprises 76 per cent corn and 20 per cent gluten. Therefore more than half of the total phosphorus is in the form of phytin. Recent

investigations by Lowe and Steenbock<sup>27</sup> have demonstrated that phytin phosphorus is poorly utilized by rats. It is readily soluble in gastric juice, and is absorbed without decomposition. It must be split before it can be utilized.<sup>29</sup> Part is reëxcreted unchanged in the feces. The lack of availability of this phosphorus simultaneously with calcium permits the independent metabolism of these two elements. This may account for the ricketogenic qualities of the cereals which Mellanby attributed to a rickets-inducing factor.

**Digestion.**—The digestion of food in the intestine by hydrolysis converts the principal part of ingested phosphorus to orthophosphate. Both this inorganic phosphate and organic phosphate are absorbed from the intestine.

**Fat.**—It has been held that the split products of fats undergo absorption through the intermediary formation of phospholipids, which are water-soluble, and subject to transport across cell membranes. They may then be resynthesized into neutral fat, in which case the phosphate is freed, or they may be metabolized.<sup>45</sup> It has been suggested that vitamin B<sub>2</sub> is essential for the phosphorylation of fatty acids, but this mechanism has not yet been proved. Obviously this is not the only mechanism in fat transport, but we cannot here enter into a discussion of the part played by the enzymes, by bile acids, cholesterol and soaps.

It is probable that the phosphatides are split before absorption, and are resynthesized by the intestinal mucosa. They enter the circulation through the thoracic duct. When injected in solution an increase in blood phospholipid is found for several hours, and then the liver content is increased. The circulatory phospholipids are not deposited in any of the body tissues except the liver and intestinal mucosa.<sup>46</sup>

**Protein.**—The metabolism of nucleoprotein starts in the stomach with the removal of purines and the conversion of the residue into soluble forms. The phosphate is split off in the intestine. The purine or pyrimidine bases united to carbohydrate are absorbed as such. Both purine and phosphate excretion are increased after ingestion of large amounts of nuclear material.

As casein digests in the stomach the phosphorus is removed very slowly. The inorganic phosphate of the chyme increases during intestinal digestion. The metabolism is that of proteins in general, except for the larger amount of phosphorus available for either storage or excretion.

**Excretion.**—The factors which determine the amounts and paths of excretion are discussed in Chapter 14.

**Urine.**—Of the total phosphorus eliminated in the urine 92-96 per cent is inorganic. A small but definite portion is present in organic form in the urine of both infants and adults—about 0.01-0.03 gm. daily. The major portion of the organic phosphorus in the blood is present as phospholipids, but these products are not found in the urine. In spite

of the fact that the blood, kidney and intestine contain phosphatases, and that the amount of hexose ester in the serum is very small, unsplit ester phosphate, especially glycerophosphate, is detectable in the urine. Ordinarily 1 mg. of phosphorus per day is present as glycerophosphate, but this may be increased tenfold after administration of drugs, especially morphine. Fasting also increases the organic phosphorus, but exercise and diet do not. The physiological significance of urinary organic phosphorus remains unknown.

*Feces.*—By far the larger part of phosphorus in the feces is inorganic, and the amount of this fraction depends primarily upon the calcium and magnesium excreted by this route. Fatty acids also combine with calcium and magnesium. The greater the amount of these soaps, the less is the amount of phosphorus present.

Little is known concerning the chemical compounds of phosphorus found in the feces. The organic compounds present may have their origin in the food residues, bacteria, or in intestinal excretion or secretion. They are not protein in nature, with the rare exception of casein curds, which are occasionally found in the feces of infants. Some of the substances seem to resemble nucleic acids. Some phosphorus is present in the lipids extracted by ether. Whether this lecithin comes from the food which is easily split, or from bile or other residues is not known. Phytin phosphorus is also found.

Rogoziński<sup>42</sup> found that with an average mixed diet human feces contained phosphorus in the following percentages of total phosphorus: lecithin, 20; phytin, 2; "protein" (organic phosphorus insoluble in dilute acid), 30; inorganic, 48.

**Ratio of nitrogen to phosphorus.**—Although it has been sought assiduously, slight relationship between the nitrogen and phosphorus metabolism has been found. When the N/P ratio of the intake is low, the excess phosphorus is excreted; when it is high, a small excretion results. When phosphorus is a limiting factor in growth, the nitrogen retention is also limited. The phosphorus is associated not only with proteins but also with carbohydrates, fats and especially bone. Therefore the interpretation of metabolism experiments is difficult either in regard to the site of storage or the source of the phosphorus excreted.

In starvation or other forms of acidotic metabolism, phosphorus and calcium are readily withdrawn from the bones. Gamble *et al.*<sup>10</sup> made calculations as to the source of the various minerals lost under such conditions. The average ratio of N/P in muscle is about 14.7, in the brain 5.0, and in the whole body, 3.2. He estimated the loss of phosphorus from muscle tissue from the nitrogen excretion. The rest of the phosphorus lost was assumed to come entirely from the bones. Because of its ubiquitous location these assumptions regarding phosphorus excretion are less satisfactory than those for other elements. When nitrogen



is being stored the phosphorus stored may be either in excess or in smaller amount than that required for muscle tissue. Forbes and Keith<sup>9</sup> have considered carefully and extensively all the evidence extant as to the variations of metabolism of phosphorus in its various combinations.

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## Chapter 8

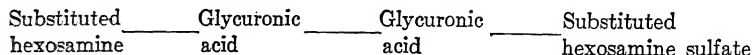
### Sulfur

The chemistry of the sulfur compounds (like that of phosphorus) and their part in body structure, functions and metabolism form an extremely complex study. The exhaustive monographic review of all the literature on sulfur metabolism, including the chemical structure of the body in health and disease, by Kahn and Goodridge<sup>22</sup> is a storehouse of the older data. Critical reviews of sulfur metabolism have been made by Lewis<sup>30, 31</sup> and by Vigneaud and Dyer.<sup>42</sup>

#### Compounds of Sulfur

Every cell of the body contains sulfur, located mainly in the proteins. Plants probably form protein from inorganic sulfates, but animals cannot. In animals however, small amounts of inorganic sulfate occur in the blood and tissues. Sulfur also occurs united to the carbohydrates and lipids. It has recently been proved that vitamin B<sub>1</sub> has a sulfur-containing nucleus, thiochrome.<sup>46</sup> A sulfur compound has recently been found in the saponified fraction of an extract of adrenal cortex of the probable formula diethanol sulfoxide.

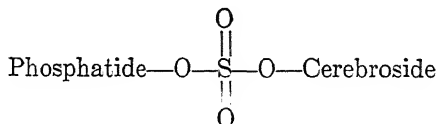
**Carbohydrates.**—Unique among known constituents of tissue are mucoitin or glutathionic acid (found in mucin, vitreous humor and cornea, serum, mucoid, ovomucoid and ovarian cysts) and chondroitin sulfuric acid (located in cartilage, tendon, aorta and sclera mucoids). They contain sulfuric acid (about 5.3 per cent of sulfur) in combination with carbohydrate, presumably in ether linkage. The probable composition of these is two molecules of glycuronic acid joined to two molecules of a substituted hexosamine in which one hydroxyl group is replaced by sulfate. In mucoitin the carbohydrate is acetylaminotalose (chondrosamine) and in chondroitin sulfuric acid it is acetylaminomannose (chitosamine). The two compounds probably differ also in the manner in which the  $\text{SO}_4^{--}$  group is attached to the carbohydrate. The chemical skeleton is as follows:



Little is known of their metabolism. Heparin, the substance which prevents the coagulation of blood, is now known to be a sulfur com-

pound of this type. Jorpes<sup>21</sup> feels that it is a mucoitin derivative rather than one combined with chondroitin.

**Lipids.**—The sulfatides which are found in the brain and nervous tissue are compounds of sulfur with lipids. These compounds were originally studied by Thudichum<sup>41</sup> and by Koch.<sup>23</sup> In recent years our knowledge of them has not been fundamentally advanced. Presumably, in these compounds, the sulfate unites a molecule of phosphatide to one of cerebroside.<sup>23</sup>



Levene<sup>29</sup> in 1912 discovered a sulfatide without phosphorus, but its physiological significance has not been studied.

**Proteins.**—The sulfur-containing amino-acids form an integral and essential part of proteins. The sulfur varies from 0.3-1.6 per cent, with an average of about one per cent (see Table 17). Abel<sup>1</sup> showed that

Table 17.—Nitrogen and Sulfur Contents of Typical Proteins.\*

Protein	Nitrogen (%)	Sulfur (%)	N/S
Legumin .....	18.04	0.38	46.9
Zein .....	16.13	0.60	26.9
Edestin .....	18.69	0.88	21.2
Gliadin .....	17.66	1.03	17.2
Leucosin .....	16.80	1.28	13.1
Casein .....	15.78	0.80	19.7
Myosin .....	16.67	1.27	13.1
Serum globulin .....	15.85	1.11	14.3
Egg albumin .....	15.51	1.62	9.6

\* From the data of T. B. Osborne, cited by Sherman,<sup>39</sup> Table 4.

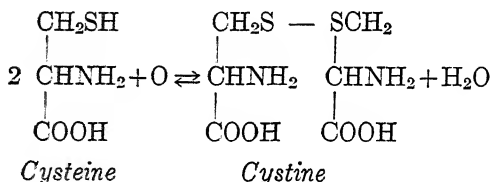
insulin is a protein which contains about three per cent of sulfur. Some of the sulfur in protein is called "loosely bound" and its exact nature and function are unknown. Presumably, it is derived from free sulphydryl groups.

The sulfur-containing amino-acids are cystine, its reduced half-form cysteine, methionine ( $\gamma$ -methylthiol- $\alpha$ -aminobutyric acid, related to cysteine) and ergothionine (thiohistidine). Osborne and Mendel<sup>35</sup> could obtain neither growth nor maintenance without adequate cystine. Both methionine and cystine have been considered essential amino-acids. Jackson and Block<sup>20</sup> showed that methionine can replace cystine, for the growth of rats, but Rose and co-workers<sup>36</sup> further proved that cystine cannot replace methionine. Cystine is not an essential amino-acid, but methionine is. When the diet contains small amounts of both,

as it does with a "synthetic" mixture containing about 8 per cent casein as the sole protein, the addition of either cystine or methionine will produce growth.

That these, together with certain non-sulfur-containing ones, complete the list of the essential amino-acids was proved by Rose. He obtained successful nutrition with known amino-acids (including threonine which he recently discovered) when they were fed as the sole source of nitrogen and sulfur.<sup>34</sup>

*Glutathione*.—The discovery of glutathione by Hopkins and its importance in the oxidation-reduction mechanism disclose a fundamental action of sulfur compounds in the body economy. This substance occurs in the blood and tissues. It is a tripeptide of glycine, cysteine and glutamic acid. The basic reaction for the oxidation of compounds of this type is illustrated by cysteine and cystine:



The oxidation of cysteine to cystine was shown by Warburg<sup>44</sup> to be actively catalyzed by traces of iron. Concentrations as low as 1 $\mu$ g. of iron per liter produce this effect. Very pure cysteine has only 1/100-1/250 of the rate of auto-oxidation of preparations previously called "pure." Harrison<sup>47</sup> found that there still remains a very slight amount of auto-oxidation due either to other catalysts or to cysteine itself.

The oxidation of glutathione is similarly catalyzed by minute traces of iron. Voegtlin showed that copper also acts as a catalyst. Harrison<sup>47</sup> was able to show that impure glutathione is auto-oxidized in a half hour, whereas the pure substance is oxidized to the same degree in six hours. Glutathione is believed to act as a coenzyme in oxidation-reduction systems (see Chapter 7, *Phosphorus*, p. 182).

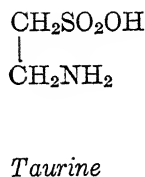
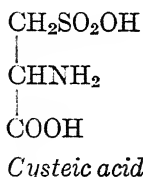
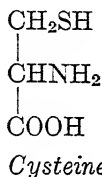
The presence of the SH-group is shown by the nitroprusside reaction. This reaction is given by glutathione and cysteine, but is not produced by cystine or native protein. The reaction is absent in the hen's egg, but after incubation for 30 hours is marked. In the absence of cystine or vitamin B<sub>1</sub> in the diet the tissues fail to give the test. Liberation of SH-groups from egg-white is affected by pepsin (in acid media), but not by trypsin (at alkaline pH).

*Cystine* addition causes an increased nitrogen balance in dogs on low protein intake.<sup>30</sup> Cystine has been said to be the limiting factor

in the production of wool. The cystine-rich tissues, wool and hair, especially red hair, contain about five per cent of sulfur.

It has been suggested by Harding and Young<sup>16</sup> that cystine is a precursor of creatine. The increased creatine excretion after cystine ingestion may be due to the acid formed when the cystine is oxidized to sulfuric acid.

*Taurine* (aminoethylsulfonic acid) is derived from cystine.



It combines with cholic acid to form taurocholic acid in the bile. Taurocholic acid constitutes about two to three per cent of the bile solids, about one-half to one-third as much as glycocholic acid. The taurine appears to be in excess of the amount of cholic acid normally present, for feeding taurine does not increase the amount of taurocholic acid unless cholic acid also is given. However, excess cholic acid administered to a fasting animal does not cause an increase in bile acids, and in the dog<sup>30</sup> may form glycocholic acid which is not present normally. It has been estimated by Garrod<sup>12</sup> that 30 per cent of all cystine metabolized forms taurine. Taurine given by mouth is poorly oxidized, and causes an increase of the neutral sulfur in the urine. Taurocholic acid is not found in the feces of adults. *In utero* the taurine is poorly absorbed and meconium is so rich in it that the sulfur as  $\text{SO}_4$  is about 30 to 40 per cent of the ash. That it is not excreted by the kidneys is shown by the fact that amniotic fluid contains only a very small amount of sulfur.

In the intestinal tract  $\text{H}_2\text{S}$ , methyl and ethyl mercaptans, thiosulfates and indoxyl may be formed from protein by the action of bacteria. Such transformation may result from the ingestion of cystine, elemental sulfur, thiourea, sulfites, thiosulfites, but not taurine.<sup>30</sup> These compounds, if absorbed into the blood stream, may be in part excreted in the urine, but Haggard<sup>15</sup> has shown the enormous capacity of the blood to oxidize  $\text{H}_2\text{S}$ . Many times the lethal dose may be injected intravenously without toxic effect. However, a rare condition, sulfhemoglobinemia, is due to long-continued absorption of such products and their combination with hemoglobin. This condition has been observed also after administration of sulfanilamide.

**Melanin.**—Melanin and the pigments of the body occur especially in epidermal structures and give color to the hair and skin. Patholog-

ically also these pigments may color the skin, as in Addison's disease. They contain sulfur and oxidation products of the aromatic amino-acids like tyrosine. Some and perhaps all of these are protein in nature. They are found pathologically in the malignant tumor, melanotic sarcoma. The rare condition, ochronosis, is characterized by pigment located principally in the cartilage. Chronic phenol poisoning may be an etiological factor. Alkaptonuria, which also occurs as an unusual anomaly of metabolism, is associated with this disease in about half of the cases. In this condition the urine turns dark on standing, due to homogentisic acid, which contains no sulfur. This is believed to be due to an error in tyrosine or phenylalanine metabolism.

Leonard<sup>25</sup> states that dioxyphenylalanine, called "dopa," is transformed into melanin by tyrosinase and "dopa" oxidase, *in vitro*. The side chain of "dopa" is formed into an indol ring and thence, with the addition of sulfur, to melanin. The mechanism of this transformation is detailed by Luck.<sup>33</sup> The formation of homogentisic acid (described by Garrod) may thus result not only from amino-acid, but also from melanin metabolism.

Most benzene ring compounds are excreted conjugated with either glycocholic or sulfate. When monochlorobenzene or monobromobenzene was fed to dogs, cystine was converted to mercaptic acid—an illustration of one type of detoxication process. The formation of ethereal sulfates represents another type. In the case of alkaptonuria, homogentisic acid (*p*-dioxybenzene acetic acid) is the end product. It is excreted as such without detoxication.

Normal serum, but not that of alkaptonurics, has an enzyme which destroys this acid. When homogentisic acid is fed to normals in large amounts, a small part may be excreted, but the acid is excreted *in toto* by alkaptonurics (as is gentisic acid, its homologue with a  $\text{CH}_2$  omitted). From 1 to 18 gm. may be excreted, which, as any other acid, increases the acidity of the urine. This condition, then, is not related to sulfur metabolism, except that it shows that all benzene ring compounds need not be detoxicated. It does afford a glimpse of the intermediary metabolism of the benzene ring acids. A fascinating account of these studies has been furnished by Garrod.<sup>14</sup>

**Thiocyanates.**—That thiocyanate is excreted in the saliva and also in the urine and other body fluids has long been known, but its significance is obscure. The amounts are very small. Presumably they are formed when the body detoxicates cyanides.

### Distribution of Sulfur in the Body

As can readily be seen from the tables in Chapter 2, the total sulfur of the body and its distribution are poorly defined. The muscles account for about one-half and the skeleton and skin about one-eighth each. The sulfur content is determined largely by the distribution of protein throughout the body, and therefore a discussion of the sulfur content of many of the individual organs has been omitted. Such a quantitative distribution of course gives no idea of the importance of the substance, for this depends upon the form in which it is found. For example,

the total hydrogen in the body does not show the significance of  $[H^+]$ , nor does the total calcium content bear any relation to  $[Ca^{++}]$ .

**Muscle.**—Sulfur constitutes about 0.25 per cent of muscle. This is distributed approximately as follows: protein sulfur, 75 per cent;\* lipid sulfur, about 6 per cent; and inorganic sulfur as high as 25 per cent.<sup>27</sup>

**Brain.**—Koch and Mann<sup>24, 25, 26</sup> gave data for the distribution of sulfur in brain tissue. Sulfur constitutes 0.50 per cent of the solids. Fifteen per cent of the gray matter and 30 per cent of the white matter are solids, but because the amount of gray matter is double that of the white matter, the total solids in the two are almost exactly equal. The percentage distribution, given in Table 18, shows that the protein sulfur constitutes about two-thirds of the total sulfur. (The protein phosphorus amounts to only 5 per cent of the total phosphorus in the brain.) The large portion is in the gray matter. The lipid sulfur is next in quantity, and the major portion is found in the white matter, whereas, of the neutral sulfur, twice as much is found in the gray matter as in the white. The inorganic sulfur is evenly distributed.

Table 18.—Distribution of Sulfur in the Brain.\*

	6 Weeks	% Total Sulfur			% Total Sulfur		
		Gray	White	Whole	Gray	White	Whole
Protein S .....	62	63	55	59	73	51	62
Lipid S .....	6	6	27	17	7	36	22
Neutral S .....	26	22	13	17	12	6	9
Inorganic S .....	6	9	5	7	8	7	7

\* Koch and Mann.<sup>28</sup>

**Blood.**—The normal values for inorganic sulfate of blood plasma vary from 0.8 to 1.7 mg. of sulfur per 100 cc. and for the conjugated sulfates from 2.0 to 3.5 mg.<sup>43</sup>

The relation of the concentration of a substance in the blood plasma to its excretion in the urine has been used both for the study of the physiology of secretion and as a measure of kidney function; sulfate has been so employed. Hayman and Johnston<sup>18</sup> have made such a study of sulfate clearances (see p. 76). They concluded that sulfate was not to be regarded as purely a secretion of the glomeruli, but showed also some reabsorption by the tubules. Further, the sulfate excretion is as good a measure of the kidney function as is urea or creatinine. In kidney disease the sulfate concentration of the blood is increased as soon as, or before, the urea concentration with progressive kidney dam-

\* Sulfur constitutes 1.27 per cent of myosin (Table 17). If the protein is 15 per cent of the total muscle, the protein sulfur equals 0.19 per cent of muscle.



age, and may reach a value as high as 35-40 mg. of sulfur per 100 cc. of serum.

The various fractions of sulfur found in the urine are undoubtedly present in blood. However the amounts found in the circulating fluids have not as yet been determined with sufficient accuracy to warrant discussion.

### Metabolism

From the viewpoint of nutrition, the sulfur intake is not a problem, provided the protein is adequate qualitatively and quantitatively. The sulfur of proteins is absorbed only slowly, for the proteins must first be broken down to the amino- acids. Cysteic acid and sulfates are absorbed more rapidly than the amino- acids.<sup>3</sup>

Our knowledge of sulfur metabolism, as of other elements, is defective because biochemists have devoted themselves largely to a study of the portion found in the urine and have too often neglected that in the feces. A study of Clark's data (see Table 40, p. 336) or that of

Table 19.—Comparison of Nitrogen and Sulfur Metabolism.\*

	Nitrogen (gm./day)	Sulfur (gm./day)	N/S
Intake .....	20.1	1.60	12.6
Output			
Urine .....	18.6	1.28	14.5
Feces .....	1.4	0.44	3.3
Total .....	20.0	1.72	11.6

\* Selected from the data of Wendt<sup>45</sup> as a typical day of one subject with an intake of 8 gm. of NaCl, and with the subject practically in nitrogen and sulfur equilibrium.

Wendt (Table 19) show that one-fourth to one-sixth of the sulfur leaves the body by the latter route. However, Grabfield and Prescott<sup>14</sup> have recently reported much smaller values for fecal sulfur in the normal subject. In one of Wendt's studies the fecal sulfur exceeded the intake. Forbes *et al.*<sup>10</sup> found that cows excreted three or four times as much sulfur in the feces as in the urine.

**Ratio of nitrogen to sulfur.**—The abundance of both nitrogen and sulfur in protein permits either to be used as a measure of protein metabolism. Further interesting information is obtained when one is considered in relation to the other. Although special proteins (see Table 17) show marked difference in their sulfur content, the usual amount in a mixed diet is about one per cent of the protein, and the ratio of N/S is about that usually found in muscle, from 14 to 16.

Wendt made a study on two individuals with a generous and also

spare protein intake, with egg-white and with varying NaCl and other salt additions.<sup>45</sup> A protocol for a single normal control day is shown in Table 19. Because of the small amount of fecal nitrogen the ratio of N/S in the feces was only about 3/1 or 4/1. The urinary ratio is usually about 14/1 or 15/1 on an ordinary diet. When the total sulfur metabolism is considered the ratio is thus lower than that for the urine alone—11/1 to 13/1. He found similar relationships when food adequate in calories but poor in protein was consumed, only here the fecal sulfur was even more prominent.

Most workers have given the N/S ratios of the urine during starvation as 10 to 16. In Benedict's careful study of a 31-day fast<sup>4</sup> the values varied within quite narrow limits over the whole period between 14 and 17, average 15.7. During long periods of fasting when no feces are passed the urine alone gives a complete picture of the protein catabolism. Thus the constancy of the ratio of N/S gives considerable evidence that, as protein catabolism proceeds, no change ensues in the nature of the materials broken down or in their intermediary metabolism. Apparently the body has no special mechanism for the conservation of sulfur, but excretes nitrogen and sulfur in constant proportions. However, on refeeding after a period of fasting, the ratio in the urine rises, indicating an extra retention of sulfur. When egg albumin, a protein high in sulfur, is given before and after fasting, the above phenomenon is even more marked.<sup>47</sup> This indicates that sulfur may be stored (at least temporarily) in combinations other than protein.

The sulfur metabolism of the growing child has been carefully studied by Schwarz.<sup>37</sup> He found 61 per cent of the ingested sulfur in the urine, 7.8 per cent in the feces and 31.1 per cent retained (as was also a correspondingly large part of the nitrogen). The ratio of N/S in the urine was 7.9. The sulfur fractions were similar to those of an adult on a high protein diet (see next section).

Grabfield has made an extensive study of the ratio of N/S excreted in kidney disease and finds that in glomerulo-nephritis with edema the body retains sulfur in excess of nitrogen. In the latter case the protein excreted in the urine may be so poor in sulfur as to have an N/S ratio of 230 while the urine consistently shows an N/S ratio of 30 or higher.<sup>13</sup>

**Sulfur partition in the urine.**—When sulfur is completely oxidized in the body, sulfate is formed and excreted in the urine. The other fractions of urinary sulfur are ethereal sulfate and neutral sulfur. Folin devised methods for determination of the fractions of sulfur and laid the groundwork for the interpretation of these values; see Table 20.

*Inorganic sulfate* usually represents about 1 gm. of S per day, and constitutes about 90 per cent of the total sulfur in the urine. It is the largest and most variable fraction, because it increases or decreases with the amount of protein metabolized. When subjects were fed Folin's

Table 20.—Sulfur Partition in the Urine.\*

	High Protein Intake		Low Protein Intake	
Urine volume .....	1170.0	cc.	385.0	cc.
Nitrogen in urine .....	16.8	gm.	3.60	gm.
Sulfur in urine .....	1.46	gm.	0.304	gm.
N/S .....	11.5		12.7	
Fractions of Sulfur	(gm.)	(%)	(gm.)	(%)
Inorganic sulfate .....	1.31	90.0	0.184	60.5
Ethereal sulfate .....	0.076	5.2	0.04	13.2
Neutral sulfur .....	0.071	4.8	0.08	26.3

\* Folin.\*

low-protein diet it dropped to one-fifth of its usual value. The counterpart of this fraction in nitrogen metabolism is urea, which likewise varies with protein metabolism.

Scott and Hastings<sup>38</sup> noted an increase in the sulfur excretion proportional to the amount of exercise. It was found that the excretion of sulfate sulfur increases with work, both absolutely and per gm. of nitrogen excreted, as compared to that of man at rest in bed.

*The ethereal or organic sulfates* consist of compounds of the type of phenol or indoxyl conjugated with sulfate. They originate from bacterial decomposition products of protein, or cyclic hydroxy- compounds which are subsequently detoxicated. A small amount (about 5 mg./day) may be a chondroitin acid fraction. Indoxyl sulfate, the potassium salt of which is called indican, has been used clinically as a measure of intestinal putrefaction.

The amount of ethereal sulfate excreted is most markedly increased when phenol and cystine are fed together. Ingestion of phenol alone or of phenol and colloidal sulfur causes an increase, but thiourea gives questionable results, and sulfates result in no increase. Lewis<sup>30</sup> suggests that this may mean conjugation before the complete oxidation of cystine. Hele<sup>19</sup> has shown, however, that sodium sulfate, sodium hydrogen sulfite or cystine, together with guaiacol (which is non-toxic), are readily used to synthesize organic sulfate. Neuberg discovered an enzyme, sulfatase, which is capable of splitting ethereal sulfates. This lends weight to the suggestion of Ambrose and Sherwin<sup>2</sup> that these compounds may have further metabolic significance.

*The neutral or unoxidized sulfur* in the urine is generally assumed to be of endogenous origin. The exact composition is not known.

The neutral sulfur is quite constant, and hence may be compared to creatinine, and each has been used as a measure of the functioning protoplasm in the whole body. It has recently been shown by Brody<sup>6</sup> that, considered over the whole range of animals from the mouse to the elephant, the neutral sulfur correlates with the caloric output, and hence can be used as a measure of basal metabolism.

Urochrome, believed to be a neutral sulfur-containing compound,<sup>8</sup> gives color to the urine, which is dark when the amount of urine is small and pale when large. This has led to the hypothesis that this is an endogenous product constant in amount. Drabkin<sup>7</sup> found that normal humans, dogs and rats excreted 42 mg./sq. m. of body surface per day. The amount increased with the rate of metabolism.

Cystine also is present in urine, but this accounts for only a small part of the total sulfur. Other fractions are taurine, bile acids, thiosulfates, thiocyanates, oxyproteic acid, uroferic acid and unknown substances.

**Cystinuria.**—Cystinuria is a rare inherent defect of metabolism in which considerable amounts of cystine are excreted in the urine. A small portion of the excreted cystine seems to be endogenous, but the larger amount is due to inability to oxidize ingested protein. Apparently the cases vary considerably in their metabolism. With increase in protein intake, cystine excretion may increase<sup>32</sup> or (according to Lewis) the individual may show an increased ability to oxidize cystine.

Ingested cystine, homocystine or methionine causes a considerable increase in excretion of cystine, whereas glutathione gives only a slight increase. This led Brand and co-workers<sup>5</sup> to state: "Although cystine excretion in cystinuria is caused mainly by dietary methionine, the inborn error in metabolism is concerned with the handling of cysteine."<sup>14</sup> However, there are cases on record in which ingested cystine is oxidized. Thiele<sup>40</sup> fed such a case 4.6 gm. of cystine which had been isolated from the patient's urine, and this cystine was completely oxidized. There is sometimes an associated condition in which diamines are also excreted. Cadavarine and putrescine are formed from the diamino-acids lysine and ornithine, derived from arginine. Leucine and tyrosine may also be found. This indicates an impairment of protein metabolism other than that affecting the amino-acids which contain sulfur. To lessen excretion of cystine, low-protein diets have been recommended, and to prevent precipitation the addition of enough alkali to make the urine alkaline. Cystine may be present infrequently in urinary calculi, where it was discovered, and whence it derives its name.

**Acid-producing effect.**—Sulfur must be considered an anionogen in studies of anion-cation relationships in intake and storage. It is partly for this reason that high-protein diets have acid-ash value. One hundred gm. of protein (a generous daily intake) have a sulfur content which is equivalent to 60 meq. of  $\text{SO}_4^{--}$ . Ingestion of this amount of protein yields 40-50 meq. of  $\text{SO}_4^{--}$  in the urine. (See also p. 293.)

$(\text{NH}_4)_2\text{SO}_4$ ,  $\text{CaSO}_4$  and  $\text{MgSO}_4$ , whether ingested or injected, act as acid-producing salts. The sulfate remains for excretion in the urine when the  $\text{NH}_4^+$  is converted to urea, or when the calcium or magnesium is excreted in the feces. These salts therefore produce dehydration and

acidosis. (See also pp. 96 and 129.) Gamble, Blackfan and Hamilton<sup>11</sup> took advantage of these properties to produce sufficient acidosis to relieve tetany in infants.

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## Chapter 9\*

### Iron

#### Functions of Iron

Iron has had an interesting history in physiology. As early as 1664 it was shown by the illustrious Sydenham<sup>64</sup> that giving chalybeate water or salts of iron to chlorotic individuals would restore pink color to their cheeks. In the eighteenth century it was shown that the ashes of many plants and food materials contain iron and that blood is unusually rich in iron. The rational use of salts of iron in the treatment of anemia was not developed until the nineteenth century, when methods for estimation of the hemoglobin concentration of the blood were developed. At the present time knowledge of the importance of iron in metabolism is largely due to the development of suitable methods for measuring the small amounts of iron which occur in biologic materials. The amount of iron present in the body of man is small; and yet iron is vitally concerned in living processes, especially with the transport of oxygen to the tissues and with the oxidation of food materials within the cells. It has been shown that the iron requirements of the body may be satisfied by ordinary foods, but inadequate intake of iron may result in anemia.

The chief functions of iron in the body are the carrying of oxygen to the tissues by the hemoglobin of the blood, and the oxidation of foodstuffs through the aid of cytochrome in the tissues. The buffer value of blood hemoglobin is an important property and it may be a function of muscle hemoglobin also. These brief allusions to the importance of iron compounds in physiology must suffice for present purposes. The subject has been reviewed by several writers.<sup>22, 29</sup> The available data serve only to emphasize the importance of iron compounds in the animal economy.

#### Iron Compounds in the Body

**Forms of iron.**—The distribution of iron in the body has been studied by observing the results of the application of appropriate reagents to sections of tissue under a microscope. In a classic contribution Macallum<sup>43</sup> has stated: "The discovery of micro-chemical methods for detect-

\*Chapter 9 was prepared by Dr. F. C. Bing, Secretary, Council on Foods, American Medical Association, Assistant Professor of Physiology, Northwestern University Medical School.

ing iron in cells has aided me in establishing the generalization that the most important of all elements in the life of every cell is an iron holding compound." Iron has been found constantly in the chromatin material of the nucleus of animal and plant cells, in the cytoplasm of non-nucleated cells, and in the prozymogen granules. Further studies with different technics have demonstrated the accuracy of Macallum's observation.

*Differentiation of organic and inorganic compounds.*—It was recognized long ago that iron in tissues exists in several different forms. Molisch <sup>47</sup> in 1891 introduced the term "masked iron" to denote those compounds that responded to tests only after treatment. Hemoglobin, for example, contains its iron in a masked form. "Inorganic iron" usually refers to that in ionogenic form, such as the ferrous or ferric salts of both inorganic and organic acids, or of protein compounds that readily yield ionized iron on proper treatment. The term "organic iron" has been used in the past to denote that which is found in complex form and which does not yield ferrous or ferric ions. Examples of organic iron compounds are hemoglobin and hematin. The terminology is not exact, however; iron citrate is usually considered as inorganic iron, yet from the point of view of the ions present, such solutions contain iron in complex form.

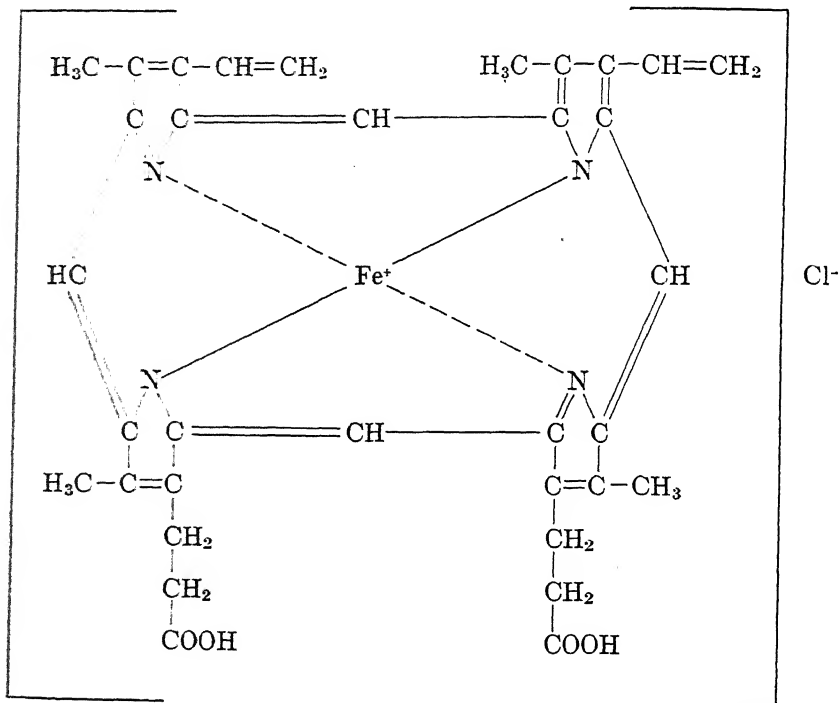
Macallum distinguished the forms of iron in tissue sections by treating them with hematoxylin; inorganic iron salts stained black, but organic iron compounds stained only after prolonged treatment with acidified alcohol. In 1930 Hill <sup>28</sup> introduced the use of dipyriddy as a reagent for the detection of minute amounts of ferrous iron. It is now customary to refer to iron which reacts with dipyriddy, after reduction with a suitable reagent, as "ionizable" or "inorganic" iron. The difference between total iron and inorganic iron gives the organic iron.

*Hemoglobin and hematin compounds.*—Quantitatively the most important organic form of iron is hemoglobin. In 1886 Zinoffsky <sup>69</sup> crystallized hemoglobin from horse blood and showed that the pure crystals contained 0.335 per cent iron. Later investigators found that pure hemoglobin obtained from the blood of different animals contains 0.30-0.59 per cent iron, depending on the species. It is probable that the higher values are inexact because of the technical difficulties involved both in the preparation of hemoglobin and in the determination of its iron. Haurowitz <sup>25</sup> found that crystalline hemoglobin from human blood contains 0.37 per cent iron. Morrison <sup>50</sup> found slightly lower values for the iron content of dried hemoglobin from human blood, namely, 0.30 per cent iron.

Both hemoglobin and other related compounds show characteristic absorption spectra. Anson and Mirsky,<sup>2</sup> by use of a micro-spectroscope, were able to detect in many different tissues the presence of an iron-



porphyrin complex, which they called haem or heme. They found it in the wing muscles of insects, in plant tissues, and in many animal tissues. They regarded hemoglobin as a special form of a universally distributed heme. Iron-porphyrin compounds have been isolated from yeast, oats and other substances. The three components of cytochrome are considered by Keilin to be heme compounds.<sup>32, 33, 34</sup> There is evidence also that catalase and peroxidase are heme compounds. These investigations have done much to clarify the problem of the nature of the iron compounds of cells.



Hemin

(After H. Fischer and K. Zeile <sup>20a</sup>)

The formula shown above represents current conceptions of the structure of hemin as the chloride of the ferric compound of 1,3,5,8-tetramethyl-2,4-divinylporphyrin-6,7-dipropionic acid. Hemin would be called ferriheme chloride, according to the nomenclature proposed by Pauling and Coryell.<sup>52a</sup> Substitution of  $\text{OH}^-$  for  $\text{Cl}^-$  produces hematin or ferriheme hydroxide.

It is possible to obtain from blood, by treatment with acetic acid in the presence of sodium chloride, quantities of hemin (Teichmann's crystals). Hemin is the chloride of a heme which contains about 8 per cent iron in the ferric condition. The structure of hemin has been established by the classic investigations of Willstätter, Küster and H. Fischer. The compound was synthesized by Fischer and Zeile in 1929.<sup>20a</sup> The molecule of hemin contains one iron atom and four methylated pyrrole groups. Splitting off the iron under proper conditions gives rise to porphyrins. The chemistry of the heme and porphyrin compounds has become a special field of investigation. For present purposes it must suffice to call attention to the structure of the compound which the body synthesizes in the production of hemoglobins of blood and muscle, and cytochrome and peroxidase of the tissues.

*Hemosiderin.*—In certain cells of the liver, spleen and kidney occur brownish-yellow masses which are demonstrably rich in iron; this material has received the name of hemosiderin. There is little doubt that hemosiderin is the iron residue of hemoglobin remaining after the destruction of red blood cells. Cook<sup>12</sup> has isolated hemosiderin from the tissues and has shown that it contains a small amount of a yellow-brown pigment, an iron moiety that gave, on analysis, 55 per cent iron, 12.5 per cent hydrogen, 27 per cent oxygen and traces (probably impurities) of carbon and nitrogen. The substance appears to be ferric hydroxide in colloidal form. Because this compound represents an important endogenous source of iron to the body, its structure therefore is of considerable interest.

*Other forms of iron.*—Little is known about the nature of the other compounds of iron in the body. Many years ago Hammarsten and Bunge isolated from ox pancreas and egg-yolk certain protein compounds which contained iron. Phosphocarnic acid of muscle is another iron compound which has not been investigated recently. Various investigators have found iron in specimens of nucleoprotein and other protein compounds obtained from tissue. The presence of small amounts of iron in nucleoproteins might be expected because the phosphoric acid groups can combine with ionizable iron. These compounds are probably loose combinations with iron; they readily give a test for inorganic iron by the dipyriddyol or similar methods.

**Quantitative distribution.**—Czerny and Keller and The White House Conference on Child Health and Protection<sup>68</sup> summarized the data on the iron content of the new-born infant. The infant is born with from 295 to 392 mg. of iron. The average value from the reported analyses is 375 mg.; this may be too high. Values for the iron content of the human fetus at different ages show that the amount of iron in the fetus is small until the seventh month of gestation. The average daily trans-

fer of iron from mother to fetus amounts to about 0.4 mg. during the first two-thirds of pregnancy. During the last third of the gestation period the iron deposition in the body of the fetus occurs at a greatly increased rate, because of the rapid formation of blood. The daily accretion of iron during this period is about 4.7 mg., or more than ten times greater than the earlier rate of growth. Coons<sup>14</sup> has shown that the maternal loss is even larger than calculations of the iron content of the fetus would indicate, because of the iron content of placental and other tissues which are produced during pregnancy.

The data on the iron content of the new-born infant indicate that about 134 mg. of the iron are in the form of blood hemoglobin. The remaining iron is present partly in muscle (39 mg.) and other tissues as tissue iron, but the bulk of it can be accounted for only as storage iron. Stearns<sup>60</sup> has reviewed the evidence concerning the iron content of the liver of the new-born infant. She concluded that little iron is stored therein even in normal infants, and, as Strauss<sup>62</sup> has emphasized, there are smaller amounts in the livers of infants born of anemic mothers. This would indicate that the amount of storage iron is much less than the values based on older analyses of the entire fetus. Bunge's theory that the infant is born with a reserve supply of iron still holds, however, because the hemoglobin of the blood itself serves as a source of iron, a phenomenon which is discussed later.

Calculations of the iron content of the different organs and total iron content of the body at different ages have been made. These indicate that about 4.5 gm. of iron are in the body of a 70 kg. man, and of this amount about 2.9 gm. are contained in the blood, 1.5 gm. in the muscle, and small amounts in the liver, spleen and remaining tissues.

*Accretion in growth.*—It is apparent that the increase in iron from birth to maturity involves the accretion of approximately 4 gm. of iron. This requires an average daily growth of iron amounting to a little more than 0.5 mg./day. Of course the actual growth in iron is not a straight-line function, but the figures give some idea of the magnitude of the daily increase. Heath and Patek<sup>26</sup> have calculated the iron requirements for growth on the basis of the estimated iron demands for hemoglobin production. Their figures are therefore somewhat lower than the rough value calculated from data on the total iron content of the body at different ages. From their calculations Heath and Patek were able to conclude that the iron requirements for growth, per unit of weight, for boys and girls, are similar until the age of puberty. The requirements for adult men are those of maintenance only, but the requirements for women are, in addition to maintenance, the requirements for periodic blood loss in catamenia, and for pregnancy and lactation. Taking these data into consideration, the conclusion was reached that anemia due to iron deficiency is most likely to occur in infants and

children of both sexes, in girls at the age of puberty and in women at about the time of menopause. These calculations are borne out by observations on the incidence of anemias due to iron deficiency.

### Metabolism of Iron

**Absorption.**—Absorption of iron occurs primarily in the portion of the duodenum immediately adjacent to the pylorus. Absorption has not been demonstrated from the stomach, and from the jejunum or ilium only when large amounts have been administered. It appears likely that simple salts of iron are absorbed in the form of ferrous iron. Ferric iron is reduced in the intestinal tract to the ferrous condition. The role of the hydrochloric acid of the gastric juice has been studied by a number of investigators. Undoubtedly acidity renders the food iron soluble, and hence ionized, but this effect is not easily demonstrated experimentally. It has been shown that even ferric oxide can be dissolved to a small extent by the action of 0.15*N* hydrochloric acid over a period of 8 hours at body temperature.<sup>41, 63</sup> Ferric salts remain soluble only in solutions of comparatively low pH; at a pH more alkaline than 2.5 the iron tends to precipitate slowly as colloidal ferric hydroxide. Ferrous hydroxide is not precipitated until the acidity becomes higher than about pH 5.0. Since the duodenal contents are slightly alkaline, after the acid chyme has become neutralized, it is evident that conditions for the absorption of iron, even as ferrous salts, are not ideal. However, metallic iron, as in the form of *ferrum reductum*, is utilized; its availability would seem to be dependent on the amount which goes into solution as a result of the action of the acid gastric juice. Where *ferrum reductum* has gained popularity as a preferred form of iron for the treatment of hypochromic anemias, it is usually administered in conjunction with diluted hydrochloric acid. Adults with iron deficiency anemias frequently have gastric anacidity; the lack of hydrochloric acid in the gastric juice may aggravate the anemic condition by possible interference with absorption of the small amounts of iron in foods. However, such patients can absorb soluble iron salts, such as ferric ammonium citrate, when administered in the usual (large) doses.

Still other factors that may influence absorption of iron are the amount of phosphate present in the duodenal contents, gastro-intestinal motility and the condition of the intestinal epithelium. It has been shown by Brock and Diamond<sup>10</sup> and others that phosphates have a deleterious effect on the absorption of iron, probably because of the precipitation of ferric phosphate. Absorption of iron is probably decreased by diarrhea and inflammation of the gastro-intestinal tract, but conclusive evidence is lacking.

**Transport.**—The amount of iron absorbed is so small that it has been difficult to determine how the iron is carried from the intestine for

further use by the body. It was formerly thought that iron was absorbed primarily as a result of the activity of the leucocytes in the villi. Calculations of the iron content of leucocytes as compared to the total amount of iron absorbed, however, show that there are not enough leucocytes in the entire body to convey the iron across the intestinal wall.<sup>42</sup>

In the whole blood of normal adults the iron content ranges from about 40 to 60 mg. per 100 cc. Nearly all this iron is present in the corpuscles and can be accounted for as hemoglobin. The amount of non-hemoglobin iron in the blood is less than 1 per cent of the total iron. This is shown by comparisons of the total iron content with the calculated iron content based on gasometric determinations of the blood pigment.<sup>30</sup>

Attempts have been made for a number of years to ascertain the nature of iron compounds, other than hemoglobin, which might be present in the blood. Studies of the iron of blood serum at first were unproductive. Abderhalden and Möller,<sup>1</sup> for example, accounted for all the iron of horse serum as hemoglobin. Some hemolysis appears to be unavoidable in the process of drawing samples of blood and obtaining serum for analysis. Even with the greatest of care, serum usually gives a positive benzidine test and shows absorption bands of hemoglobin if a sufficiently thick layer of solution is examined.

It has now been demonstrated that there is some inorganic iron in plasma or serum. Quantitative estimations of the hemoglobin iron and of the total iron have shown that the non-hemoglobin iron of normal dog serum, for example, varies from about 0.10 to 0.20 mg. per 100 cc., and averages about 0.16 mg. The values found in human serum are usually somewhat lower. Moore and his collaborators<sup>48</sup> found from 0.09 to 0.17 mg. (average 0.12 mg.) of iron per 100 cc. of serum in 15 men and from 0.06 to 0.14 mg. (average 0.10 mg.) of iron per 100 cc. of serum in 15 women. Dipyrldyl reacts only with ferrous iron, and the test is negative with blood serum unless the serum has first been treated with a reducing agent such as sodium hydrosulfite. From these observations it is evident that the traces of inorganic iron which exist in normal blood serum are entirely in the form of ferric iron.

Older methods for determination of non-hemoglobin iron of serum involved precipitation of the serum proteins, and likewise removal, with trichloroacetic acid, of minute traces of hemoglobin which are usually present, and determination of the total iron of the filtrate. Tompsett<sup>65</sup> has shown the importance, in this procedure, of reducing the iron to the ferrous condition before removing protein. If the iron is not reduced, a portion of it may be precipitated along with the serum proteins, and will give low values. The iron content of the filtrate may be determined with dipyrldyl or, after ashing, by one of the modifications of the thio-cyanate method. Another method for estimation of the serum iron has

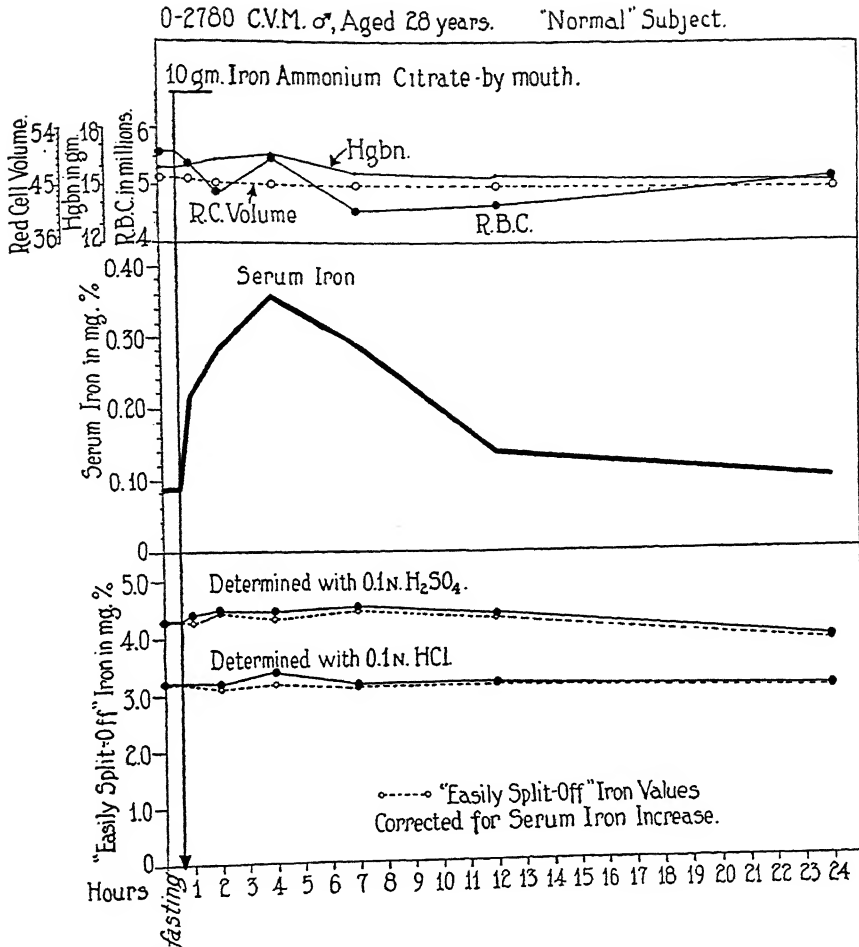


FIGURE 11. Serum Iron and "Easily Split-off" Iron Curves During a Twenty-four Hour Period Following the Ingestion of 10 Grams of Iron and Ammonium Citrate.\*

\* Figure 2 of Moore, Doan, and Arrowsmith.<sup>49</sup> Reproduced by permission of the *Journal of Clinical Investigation*.

The iron absorption is clearly reflected in the serum iron fraction. The only increase in "easily split-off" iron occurred as a result of the serum iron increase (note corrected values).

been described<sup>7</sup> which involves (1) determination of the total iron after ashing the serum and (2) estimation of the hemoglobin in another portion of the serum by means of the quantitative benzidine method. The difference between the two values gives the non-hemoglobin iron. Only 3 cc. of serum are required for single determinations of both total iron and hemoglobin iron.

Experimental studies with dogs<sup>8</sup> have shown that the concentration of serum iron is ordinarily constant for individual animals in the fasting condition. Following the administration of inorganic salts of iron the [Fe] in the blood serum increases. Values which are three to four times the fasting level are obtained, with peak values in about two hours. The concentration then decreases slowly and returns to the fasting level within several hours, the time depending on the dosage. These observations have been confirmed by studies on man, where the same type of curve is obtained<sup>49</sup> (see Figure 11). It is thus apparent that iron is absorbed from the gastro-intestinal tract and transported in the blood stream as serum (plasma) iron. Studies of the absorption of iron salts were made<sup>54</sup> on dogs in which a partial gastrectomy had been performed, so that the gastric contents were lacking in hydrochloric acid. In these fundusectomized dogs, ferrous chloride was found to be absorbed at almost the normal rate, and little improvement resulted from the simultaneous administration of hydrochloric acid by mouth.

The serum iron represents not only that which has been absorbed from the intestine and is being transported to the tissues, but also that which is derived from the breakdown of hemoglobin and other compounds in the body and is being transported to the erythropoietic system or to the tissues. It has been estimated that about 90 mg. of iron are liberated each day by the normal breakdown of erythrocytes. Because the amount of iron in food rarely amounts to more than about 15 mg. per day, it is evident that the metabolic iron, as that obtained from endogenous sources may be called, is quantitatively of prime importance in the economy of the body.

The fasting level of serum iron is probably a measure of the activity of the processes involved in the intermediary metabolism of iron, especially the production of hemoglobin. Low values for serum iron have been reported in patients after severe hemorrhage and in persons with simple anemia associated with gastric anacidity and infectious diseases. High values have been obtained after slight hemorrhage.<sup>27, 49</sup>

**Excretion.**—Histologic examinations have shown that iron is excreted chiefly by way of the large intestine and the cecum. Voit<sup>66</sup> also demonstrated the excretion of iron by analyzing the contents of isolated intestinal loops in dogs. Finally, iron salts injected intravenously lead to an increased excretion in the feces, and practically no change in the urinary iron.

The amount of iron excreted by way of the intestinal tract varies ordinarily with the amount present in the food. In fasting, about 7 to 10 mg. of iron were excreted daily by Cetti and Breithaupt.<sup>36</sup> Meconium contains a total of about 2 mg. of iron, or about 13 mg. per 100 gm.<sup>35</sup> The amount of iron in the stool is lowest when diets, adequate in all

respects except iron, are fed. Lintzel was able to establish equilibrium, for a short period, on an intake of less than 1 mg. of iron daily.

Studies of the amount of iron excreted by way of the kidney have shown that very little is removed by this channel, although variable reports have appeared regarding the precise magnitude of the urinary iron. Earlier values of iron in the urine, 1 mg. or more per day, are undoubtedly too high. Lintzel<sup>40</sup> came to the conclusion that no iron whatever is excreted by the normal adult kidney and he attributed the earlier results to faulty analytical methods. More recently it has been shown that the daily urinary iron excretion of normal adults is from about 0.10 to 0.30 mg., occasionally more.<sup>4</sup> The source of this iron has not been demonstrated but probably it is derived from the inorganic iron of the blood plasma. Some of it may be present in the epithelial cellular debris.

**Intermediary metabolism and storage.**—It now appears likely that iron is absorbed as ferrous salts and transported as ferric iron of the plasma, but what are the interrelationships of iron in the body?

Mention has already been made that iron from endogenous sources is greater than that ingested. The destruction of red blood cells is a continuous phenomenon. The old erythrocytes are engulfed by the cells of the reticulo-endothelial system in many parts of the body, especially in the endothelial cells of the splenic pulp and Kupffer cells of the liver. The prevailing opinion is that hemoglobin is decomposed into globin and hematin. The latter is further broken down into iron-free pigments that finally yield biliverdin,<sup>38</sup> which in turn is converted to bilirubin and eliminated by way of the bile. The iron remains as hemosiderin. Measurement of the amount of bilirubin excreted into the bile duct gives some indication of the extent of blood destruction. It has been observed that about 3.3 per cent of the total blood hemoglobin is destroyed daily in mammals, including man. At this rate a 70-kg. adult would break down about 29 gm. of hemoglobin per day or about 90 mg. of iron.

Iron from whatever source is retained most tenaciously by the body. Balance studies on patients with polycythemia have demonstrated this most forcefully. When phenylhydrazine and related compounds are administered to these patients, many of the red blood cells are destroyed, the number depending on the dose; and large quantities of porphyrins and possibly other organic products from the decomposition of hemoglobin are eliminated. But the body retains practically all the liberated iron.<sup>5, 56</sup> When salts of iron are injected parenterally large amounts of iron are deposited in the liver. Excretion by way of the large intestine is slow. Because of this limited ability to excrete iron it has been suggested<sup>44</sup> that the body controls the metabolism of iron by regulation of the amount absorbed rather than the amount excreted.

Blood destruction occurs in a widely scattered system of cells, but



blood formation occurs chiefly in the red bone marrow. There must be considerable traffic in iron from endogenous and exogenous sources to the site of hemoglobin formation. The principal change is that of inorganic iron compounds into hemoglobin, but the work of Greig<sup>23</sup> indicates that the reverse process may occur in which the blood hemoglobin serves as a reserve for the formation of iron nucleins of the cells. Greig's study was done with salmon during their spawning migration. Analyses of the muscles, liver and sexual organs showed a considerable transfer of iron during the development and maturation of the eggs. Only a portion of the iron could be accounted for by the breakdown of muscle tissue; none apparently came from the liver. The fish do not eat on their journey from the ocean to fresh water, and therefore the only important source of iron could be the blood. Recent observations, that in the new-born infant the hemoglobin of the blood is an important source of iron for growth, have already been mentioned.

*Depots.*—Many experiments show that the liver acts as the principal depot for iron. Its high content becomes depleted on a low-iron regimen. Probably there is a level below which the iron content cannot fall without injury to the cells. On administering iron, the content of the liver is increased. Whether the muscles can store iron is not definitely known except that variations in the iron content are probably small. Whipple<sup>67</sup> has shown that the muscle hemoglobin remains at a definite level, no matter how low the hemoglobin of the blood may fall. This he interprets as a physiologic necessity because otherwise the muscles, particularly those of the heart, could not function.

### Hemoglobin Production

The researches of Bunge and his students<sup>11</sup> from about 1880 to about 1905, to which some mention has been made already, focused attention on the problems of iron metabolism. As a result of their analyses of the content of iron in the bodies of animals at different ages they arrived at fundamental conclusions regarding the general problem of the importance of iron in nutrition.

They found that the total content of iron in the bodies of the new-born was slightly increased during the suckling period, but the percentage of iron became greatly decreased. On comparing the mineral composition of the new-born animal with the composition of the milk of the same species, an essential similarity was observed in the relative proportion of each element in milk and in the body, except for iron. Milk is a food which contains very little iron. The new-born animal contains a much higher percentage and, indeed, a greater amount of iron than the adult. It was believed that the mammal was born with a reserve supply of iron which was utilized for the necessary production of hemoglobin and other compounds during the rapid growth of the suckling period. Even before

actual weaning had begun, the iron reserves of the body were largely depleted and nutritional anemia developed. This condition could be overcome by feeding natural foods that contain much more iron than does milk, or the anemia could be made more severe by maintaining the animals on a diet consisting solely of cow's milk.

These early conclusions of Bunge and his students, particularly Hausermann and Abderhalden, were valid for rabbits, kittens and puppies. An exception was found in the guinea pig, an animal which appears to be born without a reserve supply of iron. But this exception tended only to increase the belief in the general application of the theory, because the guinea pig nurses for only a short time, and the new-born animal is able to take care of itself and to eat "extra" foods beginning almost on the day of birth. Data obtained by Hugounenq and others, to which reference has already been made, indicated that the human infant also is born with a reserve store of iron. Much of this older work has been reviewed by Meyer<sup>45</sup> and by others.<sup>11, 35</sup>

There were, however, more problems introduced than answered during this era. For example, there was no clear explanation for some of the experimental evidence which seemed to show that inorganic iron is not used by anemic animals. The theory was also advanced that organic iron compounds were superior to inorganic iron for hemoglobin production. Later research has shown these conclusions to be erroneous or only partly true.

**Role of copper.**—In 1928 Hart and his collaborators<sup>24</sup> reported some experiments which went far to explain the inability of Abderhalden and others to cure the anemia of animals on an iron-deficient diet. They found that minute amounts of copper are necessary along with iron for hemoglobin production. (For fuller discussion, see *Copper*, page 239.) Copper is not present in the hemoglobin molecule, nor is it concerned with iron absorption, but it is a "catalyst" for the transformation of inorganic iron into hemoglobin. Considerable experimental evidence has been accumulated to show not only that copper is necessary for the utilization of iron, but that this action is specific. Copper is needed in small amounts only, and an adequate amount may be supplied to anemic animals as a contaminant of the iron salts, as an ingredient of foods, or from that previously stored in the liver.

**Hemoglobin concentration in the blood.**—The normal hemoglobin content of the blood of man varies with age, sex, and to some extent with geographical location. In the new-born baby the hemoglobin concentration in the blood is about 22 gm./100 cc. Under ordinary conditions of feeding, this value drops rapidly in the first two weeks of life, then more slowly until about the age of 4-6 months, when low values of 9-13 gm./100 cc. are found. The hemoglobin concentration may remain at about this level for the next two years. In the case of babies born

prematurely or in the case of multiple births, the initial level at birth is about the same as in normal babies, but the decline is more rapid, and without treatment anemic levels may be obtained in about three months or less. As a result of the administration to infants of foods other than milk, such as egg-yolk, puréed liver, strained fruits and vegetables, or salts of iron, the hemoglobin level increases slowly during infancy and childhood. In adolescence normal values of about 14-17 gm. of hemoglobin per 100 cc. of blood are observed. The average value for men in the United States is about 15.6 gm./100 cc.; for women the values are about 10 per cent less than those found in men. That this is a sex difference and not the result of a possibly lower intake of iron is indicated by the fact that the number of red blood cells is also lower in women; the concentration of hemoglobin per cell is about the same in both sexes.

Geographic location may also be a factor in determining what is called the normal level of hemoglobin of blood. The hemoglobin level and red-cell count are increased by long-continued low barometric pressures, *e. g.*, at high altitudes. This represents a compensatory reaction by the body in response to the lower concentration of oxygen in rarefied air. Other factors probably affecting "normal" hemoglobin values are heredity, race and diet.

### Anemia in Man

Anemia means literally "without blood," but it is a term applied to conditions involving subnormal hemoglobin levels of the blood. The alterations of the status of the blood are evaluated by the determination not only of the hemoglobin content but also of the number of red cells and their average size and morphologic character. An anemic condition is not usually considered to be present unless the level of hemoglobin concentration in the blood is below 10 gm./100 cc. Values as low as 5 gm., or even less than 2 gm., have been reported. The differential diagnosis of the anemias is a problem for a text on hematology or on medicine. In general there are two types of anemia, that which is amenable to iron therapy and that which is not. In the latter group the most conspicuous example is pernicious anemia. Minot and Murphy<sup>46</sup> have shown that pernicious anemia can be treated successfully by feeding large amounts of liver or aqueous extracts of liver. The active substance is an organic compound, probably a peptide, which normal persons are able to produce by gastric digestion of muscle tissue. Although iron alone is of no value in the treatment of pernicious anemia, it is sometimes important to administer it along with liver, if the individual patient needs iron.

Those types of anemia<sup>16</sup> which are amenable to iron therapy are the hypochromic microcytic anemias, so called because the red cells contain less than their normal content of hemoglobin per cell and because

they are smaller than normal in size. This is the type of anemia observed in infants maintained on a milk diet, unsupplemented with iron-rich foods, for too long a time. Iron deficiency anemias may also be observed in chlorosis in pubescent girls, and in the hypochromic anemia occasionally encountered in middle-aged women. The anemia resulting from severe hemorrhage is also curable by iron.

The hemoglobin concentration of the blood may fall considerably without danger to life. Presumably this occurs after the iron reserves of the body have become depleted, unless there is actual bleeding. It is interesting to note that there is a large factor of safety in the normal hemoglobin concentration of man. This is indicated by studies of the amount of oxyhemoglobin in arterial and in venous blood. In the arterial blood of a resting man, for example, there may be 15.6 gm. of total pigment per 100 cc., and the concentration of oxyhemoglobin may be about 14.7 gm. In the venous blood of such a person there may be 11.7 gm. of oxyhemoglobin, and 3.9 gm. of reduced hemoglobin per 100 cc. Not all the blood pigment enters into the transport of oxygen at one time. It has been estimated that an anemic person with only 6 gm. of total pigment per 100 cc. still has sufficient pigment to permit normal arterial-venous oxygen differences, unless exercise increases the demands.

**Iron therapy.**—The rational use of iron salts in the treatment of anemias dates back to the early years of the 19th century. In 1831 Blaud<sup>9</sup> introduced his preparation of ferrous iron which was used in large doses for treatment of simple anemia. Although the use of iron fell into some discredit toward the end of the last century it has now become re-established. It is usual to administer daily, in divided doses, as much as 1 gm. of iron in the form of saccharated ferrous carbonate in the treatment of iron deficiency anemias. Other salts of iron are also used.

Some clinicians have suggested that the dose might well be decreased, because of the effect that large amounts of iron might have on phosphorus metabolism. It has been shown with rats and chickens that huge doses of iron salts, added to a normal diet, will result in the production of a type of rickets which is not amenable to vitamin D therapy. Apparently the large amounts of iron salt precipitate phosphate in the intestine, and thus affect the metabolism of phosphorus (and calcium) to such an extent that administration of vitamin D will not correct the deficiency. There seems to be no reason why untoward effects should appear in man, unless large doses of iron are continued for too long a time. With infants it might be well to exercise caution because of the possibility that interference with normal calcium and phosphorus metabolism, even for a short period of time, such as three to six weeks, might be harmful. Obviously, the matter should be investigated further and a decision reached as to the appropriate dosage under various conditions.

Because of the evidence that iron salts in the alimentary tract are reduced to the ferrous condition before absorption, it has been thought that ferrous salts would be preferable to ferric salts in therapeutics. Evidence of the superiority of ferrous salts is meager. The important considerations in the choice of an iron preparation are solubility and relative freedom from irritating effects upon the gastro-intestinal tract.

There has been a considerable amount of work done in recent years on the question of whether copper is necessary for the proper utilization of iron by the human being. The results are somewhat contradictory. Duckles, Willis and Elvehjem<sup>17</sup> have found that copper is not necessary as an adjunct to iron in the treatment of anemia in adults. There are some reports which claim that better results are obtained in the treatment of anemic infants with iron and copper than with iron alone. The amount of copper involved is small, and it is likely that some infants have enough copper in their body stores or secure enough from their food to enable them to use copper-free salts of iron.

### Balance Studies and Requirements

Quantitative studies of the intake and excretion of iron are of importance because they show the amount of iron retained during growth, they illustrate the effects of other constituents of the diet on absorption and excretion, and they indicate what the requirements are at various ages under differing conditions.

*Men.*—In the adult male, the problem of maintenance alone is involved. Studies were made by Stockman and Greig in 1897, Wendt in 1905, Sherman in 1907, and by a number of later investigators. In the experiments of Stockman and Greig the amount of iron in the food was as little as 5.6-6.2 mg. daily. On such intakes three men showed excretions of 6.3, 9.3 and 11.5 mg. per day; in other words only one of these individuals was in iron equilibrium. Wendt found excretions of 6-18 mg./day, and Sherman found fecal eliminations of 5.5, 8.7 and 12.6 mg. daily when the diet contained 5.7-7.1 mg. From these data, and from other data showing that 6-16 mg. appears to be the amount of iron eliminated per day by persons eating their customary foods, Sherman concluded that the requirements were about 10 mg./day, and that 15 mg. might be considered as a standard allowance for the adult.

More recently the problem of iron metabolism has been given greater attention with a view to determining the probable daily requirements. Attention has already been directed to the work of Lintzel, who showed that an adult could maintain iron balance for short periods of time when the intake was as low as 1 mg. of iron per day. Farrar and Goldhamer<sup>20</sup> in 1935 reported data which seemed to show that the iron requirement of adults is not more than 5 mg./day. One 26-year old man lived for 316 days on a diet which provided only 4.9 mg. of iron per day. Balance

studies performed on selected days showed that this subject was in approximate iron equilibrium. Two men were in iron balance over periods of four and five months when the daily iron intake amounted to 7.1 and 7.8 mg. respectively. The data of Farrar and Goldhamer show that the adult organism can become adapted to low intakes of iron and eventually will attain equilibrium on diets containing as little as 5 mg. of total iron per day. This low intake should be considered as evidence of the ability of the body to economize on its supply of iron rather than as a desirable intake.

*Women.*—For women, the iron requirements include the quota for maintenance and the additional demands of menstruation, pregnancy and lactation. Ohlson and Daum<sup>51</sup> have studied the iron metabolism of three normal women on self-chosen diets during 6 periods of from 5 to 15 days in length. They observed no relation between the retention of iron and losses during menstruation. No relation was observed between the balance of either nitrogen or copper and the balance of iron. Leverton and Roberts<sup>39</sup> studied the iron metabolism of normal young women during consecutive menstrual cycles. Four subjects were studied for 5-day periods during three to five months. The average daily intakes of iron were 10-13.6 mg. and the balances were from -0.2 to +1.5 mg. They found no cases of negative balance when the intake of iron was 0.23 mg./kg. of body weight per day, or more. The average menstrual losses per period were only 11.1-22.8 mg. of iron.

Coons<sup>13</sup> studied 23 iron balances of women at different stages of pregnancy. With intakes varying from 9.7 to 19.5 mg. of iron per day the retentions varied from +0.9 to +7.0 mg. with one exception, a negative balance of -2.2 mg. She concluded that, with good diet and good health, the maternal organism could assimilate enough iron from food during the period of pregnancy to supply the fetus with the necessary amounts of this element and maintain the integrity of the stores. Slight upsets in digestion seemed to be an important factor in iron retention.

*Infants and children.*—Children require iron for growth as well as for maintenance. Balance studies on infants and children have been reported by a number of investigators within recent years and some data are now available for infants up to the age of one year, and for children from three to eight years of age.

In general it has been found that under ordinary conditions of feeding, iron balances of children are only slightly positive. To secure adequate retention of iron it has been concluded that the diet should provide about 0.6 mg. of iron per kg. of body weight for children within the pre-school age group, or early school years.<sup>3, 15, 37, 53, 58</sup> This would mean that a suitable allowance of iron for a 6-year old child weighing 20 kg. would be about 12 mg., or the same amount as for the adult.

Balance studies on infants not only have shown the probable requirements, but also have served to emphasize the importance of some of the physiologic factors of blood destruction and formation during early life. The very young infant ordinarily excretes more iron than it receives in the food; that is, it is in negative balance. A large store of iron in the body of the new-born child appears to be the hemoglobin of the blood, which is at a considerably higher level than in the adult. The concentration rapidly diminishes, due in part to destruction of some of the hemoglobin, and the iron so released serves as a source for tissue growth and further hemoglobin production. Stearns and Stinger<sup>61</sup> and Josephs<sup>31</sup> showed that infants fed on human milk remain in positive iron balance, whereas those fed on cow's milk modifications usually are in negative balance, unless the diet is supplemented with an iron-containing food. Suitable intakes for the artificially fed infant, up to the age of one year, are about 1 mg. of iron per kg. of body weight, and if the intake is increased to 1.5-2.0 mg./kg., larger retentions of iron are obtained.<sup>31a</sup>

### Sources of Iron in Food

There have been many analyses of the iron content of foods; tables of average composition of representative foods are available in textbooks on nutrition and dietics. (See Table 22, p. 241.) Recent workers have called attention, however, to the discrepancy between actual iron content, as determined by modern methods, and the usual values reported in compilations of analytic data. Frequently it is found that the iron content actually is somewhat less than the reported figures by as much as 30 per cent. In general, however, it can be stated with confidence that the total iron content of foods is highest in green leafy vegetables, in beans, peas and legumes, in muscle and glandular meats, and in eggs. Parsley is rich in total iron content, but parsley is used more as a decoration than as a food; other green leafy vegetables are more valuable sources. Among meats, liver is particularly high in iron, and as is well known, it also contains the anti-pernicious anemia factor. Egg-yolk is an excellent source of iron and because it usually is well tolerated it is useful in the feeding of infants.

There is scarcely a single food which is rich enough in iron to provide an adequate intake for a day in a single portion. Small quantities of iron are supplied by each food that we eat. The potato contains only a small amount of this element, but in the quantities that are ingested, this vegetable is a considerable source of iron. Because whole-wheat bread contains about twice as much iron as white bread, it has been suggested that persons could well afford to develop a liking for bread in this form because of its iron content alone.

*Organic vs. inorganic iron.*—That there is a difference in the availability of different forms of iron has been recognized for many years.

Blood, for example, is a tissue rich in iron, but the body cannot utilize its iron when administered by mouth. The iron of blood is in the pyrrole nucleus, firmly bound in organic form. Elvehjem was able to demonstrate that anemic rats do not recover when, together with salts of copper, solutions of hematin are administered.<sup>18</sup> Because the iron in plant and animal tissues which is organically bound is largely, if not entirely, in the form of hematin compounds, it has been concluded, on the basis of such experiments, that inorganic salts of iron are absorbable, whereas organic forms of iron (hematin compounds) are of no value.

Methods have been devised for determining the relative quantities of inorganic, or ionogenic iron and organic, or hematin iron in foods; and it has been suggested that human requirements and food sources of iron be stated in terms of so-called available iron. Elvehjem and his collaborators<sup>19</sup> have been able to check on the accuracy of their analytic method by simultaneous bio-assays of a number of foods. They have reported that only about 50 per cent of the total iron of wheat is in a form available to the body for hemoglobin production. The results of Rose and others,<sup>57</sup> however, show that the iron of wheat is well utilized. Free and Bing<sup>21</sup> have slightly modified the method for the determination of available iron and have found that about 80 per cent of the total iron of whole wheat is available. The same relative value was obtained by comparing the response of anemic rats to wheat and to salts of iron. The total iron of white bread, small though this may be, has been found to be just as well utilized as the iron of inorganic iron salts.<sup>55</sup>

The observations of the availability of iron in anemic rats have been applied to the human being by Bing, Benes and Remp.<sup>6</sup> These investigators studied the utilization of hematin iron and found that some subjects did not utilize iron in this form, and that others did. Utilization appears to be dependent on the decomposition of hematin by intestinal bacteria, and possibly on other factors. These results indicate that some of the so-called organic iron of foods may be utilized by some persons and not by others. From the point of view of practical dietetics it would seem satisfactory to consider only the total iron of dietaries, at least for the present. The dietary standards that have been established have been based on analyses of foods for total iron. It should be noted in addition that Sherman, as a result of recent investigations by numerous workers, has been led to reduce the standard for a suitable daily allowance for an adult from 15 mg. to 12 mg. of total iron daily.<sup>59</sup> On the other hand, if rich sources of food iron are desired, it would be well to select products which are known to be well utilized, such as, for example, kale rather than spinach, or to administer salts of iron.

Milk is a notoriously poor source of iron. The older methods gave values of about 2 mg. of iron per liter of cow's milk. Improved methods,



however, show that the iron content may be considerably less than this value. Figures obtained for fresh cow's milk are in the neighborhood of 0.2-0.5 mg./l. In some samples of milk the iron content is actually less than the copper content, small though the latter may be. The iron content of human milk has been reported to be higher than that of cow's milk. Goat's milk, which has become well-known for its relationship to nutritional anemia in infants, has an iron content approximating that of cow's milk. It has been shown by experiments with rats that goat's milk does not contain any protein or nutritional deficiencies other than its low content of iron and copper, which cause anemia in experimental animals.<sup>52</sup> Experiments have shown that it is not possible to increase the iron content of milk by the administration of iron salts to lactating animals or women.

It has been shown that iron added to food products may in time bring about the destruction by oxidation of some of the important organic factors present in foods. Thus the addition of ferric salts causes the catalytic oxidation of vitamins A and E. Caution is therefore necessary before adding appreciable amounts of salts of iron to food products. It should be ascertained first that in the amounts added and under the ordinary conditions of storage, important organic factors are not destroyed.

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## Chapter 10\*

### Iodine

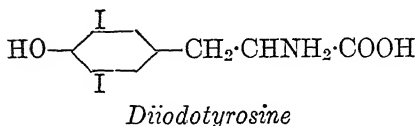
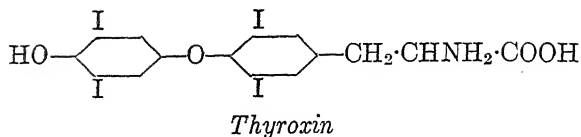
The discovery of iodine in Chilean saltpeter was reported by Gay-Lussac in 1813. Soon afterward Humphry Davy showed that iodine was present in certain seaweeds. Iodine was found in sponges also. Because of the use by the ancient Greeks of burned sponges for the treatment of goiter, salts of iodine were tried therapeutically as early as 1820 by Courtois and others. Some good results were reported; but, as Orr and Leitch<sup>23</sup> have pointed out, the use of iodine soon fell into disrepute, partly because toxic symptoms were induced by the large doses of iodides then employed, and partly because the rationale of iodine therapy had not yet been demonstrated. In the middle years of the nineteenth century, Chatin made a large number of fundamental contributions to the physiology of iodine. He demonstrated the widespread distribution of iodine in soil, drinking water and foods, always in minute quantities. He showed also the relationship between some cases of goiter and deficient intakes of iodine. But Chatin's contribution fell on barren soil. It was not until later, when clinicians were able to differentiate between various types of thyroid diseases, as due to excess or deficiency of thyroid activity, that the significance of iodine in the body became apparent.

#### Iodine Compounds in the Body

Iodine is of unique physiologic importance because of its occurrence in thyroxin, the secretion of the thyroid gland which exercises control over the rate of energy metabolism in the body. The first definite evidence that iodine is a normal constituent of tissues was obtained by Baumann<sup>1</sup> in 1895. He showed that iodine is a normal constituent of the thyroid and that the amount is diminished in simple or endemic goiter. In 1915 Kendall<sup>15</sup> succeeded, where many predecessors had failed, in obtaining in crystalline form the active constituent of the thyroid. This material contained 60 per cent iodine, and Kendall called it thyroxin. The structure of thyroxin was demonstrated by the brilliant researches of Harington; and in 1928 he, with Barger, succeeded in synthesizing this compound.<sup>12</sup>

Thyroxin is a tetraiodo- derivative of a compound of tyrosine and phenol. The formulas for thyroxin and for diiodotyrosine are as follows:

\*Chapter 10 was prepared by Dr. F. C. Bing, Secretary, Council on Foods, American Medical Association, Assistant Professor of Physiology, Northwestern University Medical School.



It is apparent from their structures that thyroxin and diiodotyrosine are amino-acids. There is evidence that thyroxin exists in a protein molecule similar to the thyroglobulin of Oswald.

The iodine of the thyroid gland has been divided into so-called acid-insoluble iodine and acid-soluble iodine. Thyroxin appears to account for all the acid-insoluble iodine of the gland, values for which have been reported as high as 70 to 90 per cent of the total iodine. Diiodotyrosine accounts for all, or practically all, the acid-soluble iodine of the gland. Harington and Randall<sup>13</sup> and Foster<sup>8</sup> have isolated diiodotyrosine from normal thyroid tissue. It is considered that diiodotyrosine is an intermediary in the formation of thyroxin by the thyroid gland. By new and more direct methods Foster<sup>9</sup> has found that thyroxin iodine accounts for only 25 per cent of the total iodine of the thyroid gland of adults. A similar value, 20 per cent, has been reported for the thyroid of the new-born by Palmer, Leland and Gutman.<sup>24</sup> These values are considerably lower than those obtained by older methods, and indicate the need for further study of the problem.

The iodine content of the thyroid gland varies with age and other conditions. In reviewing the evidence available in 1932, the White House Conference<sup>31</sup> reported that normally the amount increases from less than 0.1 mg. in that of the new-born infant to about 15 mg. in the adult. More recent figures have given lower values for the iodine content of the normal thyroid. Kolnitz and Remington and their collaborators<sup>17, 28</sup> reported an average of 8.8 mg. for the total iodine content of the thyroid glands of 150 persons who had lived in Charleston, South Carolina. In endemic goiter, the amount of iodine in the thyroid is much less than normal; it may be less than 1 mg. Iodine is found elsewhere in the body, but only in minute traces. The blood [I] is about 15  $\mu\text{g.}/100\text{ cc.}$  The total iodine content of the adult body may be roughly estimated as about 20 mg., of which approximately 10 mg. are in the thyroid gland and 10 mg. in the rest of the body.

### Metabolism of Iodine

There is considerable evidence that iodine is absorbed from the intestinal tract regardless of the form in which it is administered. The iodine of foods is probably absorbed in the alimentary tract as iodides and thus circulates in the blood. Thyroxin itself is absorbed from the alimentary tract and gives rise to its characteristic effect when so administered. Feeding experiments with animals have shown that iodized fatty acids may be absorbed and deposited as such or utilized by the body.

After the administration of iodides, the iodine content of the blood is greatly increased. Marine has shown that the thyroid is unique in its ability to abstract iodine from the blood stream. Perfusion of the thyroid for from one to two hours was followed by a tenfold increase in its iodine content. Under similar conditions, perfusion of the kidney or spleen resulted in no fixation of iodine. The iodine is converted into thyroxin in the thyroid gland, but the transformation requires some time. The excess iodine of blood, which is not fixed by the thyroid, is quickly carried around the body, distributed to the various fluids and eliminated.

Under normal conditions, most of the iodine eliminated by the body is excreted in the urine. Some may be excreted by way of the intestinal tract, particularly in diarrhea. Iodine also is eliminated in milk and in the sweat; in balance studies, it is necessary to take these losses into consideration. It is also found in saliva and tears. The amount of iodine excreted depends, of course, largely on the intake. The daily urine specimens of persons living in sections where goiter is common contain less iodine than normal. More iodine is excreted after exercise; hence the daytime urine contains more iodine than night urine.

When thyroxin is administered, the basal metabolic rate increases gradually. Continued dosages of 1 mg. have produced a rise in metabolism, and 2 mg. may increase the basal metabolism of adults as much as 20 per cent. After the administration of thyroxin is discontinued, the heat production of the body declines slowly over a period of ten days. From the curve of declining heat production, Plummer and Boothby<sup>25</sup> have calculated that thyroxin is destroyed in the body at the rate of 0.2-0.4 mg. per day. This is equal to 0.13-0.26 mg. of iodine. From the results of balance experiments, to be described, it is known that an adult may maintain iodine equilibrium with a daily intake of about 15  $\mu\text{g.}/(0.015 \text{ mg.})$ . It is evident, therefore, that nearly all the iodine liberated by the normal breakdown of thyroxin must be retained by the body.

Foster and Gutman<sup>10</sup> have studied the metabolic fate of diiodotyrosine in rabbits. When given by mouth, it is excreted in the urine largely

unchanged. About 10 per cent appears as inorganic iodide and 18 per cent as 3, 5-diiodo-4-hydroxyphenyllactic acid.

### Disorders of the Thyroid Gland

Although no attempt will be made to discuss the pathology of the thyroid gland, disorders of this gland are so intimately connected with thyroxin production and also with iodine metabolism that they disclose the physiological activity of this element. Differentiation of the various types of goiter requires expert diagnosis and treatment.

Diseases are of two types, depending on excess or deficiency of the thyroid secretion. The hyperthyroidism called Basedow's disease or exophthalmic goiter occurs especially in adolescent girls. It is characterized by symptoms of emotional excitability, rapid heart rate, fine tremor, excessive sweating and by the two symptoms which give rise to its name, namely protuberance of the eyes and swelling of the neck. There is also a loss of weight due to the augmented energy production; the basal metabolic rate may be increased to more than 50 per cent above normal. Another form of hyperthyroidism is that produced by adenomata.

Hypothyroidism causes myxedema, or if severe and congenital, cretinism. The symptoms are lethargy, often accompanied by lessened mental capacity, rough skin and coarse hair. The boggy condition of the skin gives the disease its name. The metabolic rate is lowered to as much as 40 per cent below normal.

So-called simple goiter, colloid or endemic goiter, shows a relatively mild deficiency of thyroid activity. The principal symptom is the swelling in the neck. It is most closely related to iodine metabolism, as is described below.

These symptoms have been given to show the opposite physiologic effects produced by variable amounts of thyroxin. (See also p. 113.)

**The iodine content of the blood.**—Many studies have been made of the iodine concentration of the blood because of the insight it was hoped such data would give regarding the activity of the thyroid gland. In healthy adults the blood iodine averages about 12  $\mu\text{g.}/100$  cc. of whole blood.<sup>5</sup> The range of normal values is 8-16  $\mu\text{g.}$ , the variations reported depending partly on the method used for the determination. In hyperthyroidism the level of blood iodine may be increased or it may be normal. High values, up to 60 and 80  $\mu\text{g.}/100$  cc. of whole blood have been reported, but in some patients the values are even lower than normal. After thyroidectomy the iodine content of the blood may be decreased if it was higher before operation, or it may be increased if the original level was lower than normal. The determination of the total iodine content of the blood has been disappointing as an aid in the diagnosis of hyperthyroidism. A more useful value is that of the basal

metabolic rate, which uniformly is elevated in hyperthyroidism and lowered in myxedema. Attempts have been made to fractionate the iodine of the blood into organic and inorganic forms, but the results are still inconclusive.

A seasonal variation in blood iodine has been reported; slightly higher values are said to be found in the summer months. An increase followed by a gradual decline to a level somewhat below normal has been found after severe exercise. This may be related to liberation of thyroxin from the thyroid gland during exercise. However, the evidence is conflicting and indicates that our knowledge of the intermediary metabolism of iodine is still far from complete.

**Endemic goiter.**—Endemic goiter is definitely related to deficient intakes of iodine. This condition can be prevented or cured by administration of iodides. The first clear evidence of the therapeutic value of iodine in the prevention of endemic goiter was reported by Marine and Lenhart in 1911.<sup>19</sup> Endemic goiter formerly was prevalent in certain restricted localities; in the United States, the goitrous regions were particularly in the neighborhood of the Great Lakes and in the Pacific Northwest. Dogs and other domestic animals also frequently showed colloid enlargement of the thyroid similar to the condition in man. Even the white-fish caught in Lake Erie had swellings on the midline of the ventral surface of the body, midway between the gills. In a brook trout hatchery in western Pennsylvania, a similar enlargement or "growth" was recorded in many of the fish, and also a high mortality. The condition of the fish was first thought to be due to bacterial infection, but Marine and Lenhart showed that it could be prevented either by adding tincture of iodine to the usual food supply, or by feeding ground whole sea fish, which are comparatively rich in iodine.

Following this first demonstration of successful prophylaxis in fish, Marine<sup>18</sup> and Kimball administered iodides to a large number of school children. In their first report they showed that endemic goiter in children could be prevented, or enlargement of the thyroid retarded, by the administration of small amounts of iodids to each child. These observations have been verified many times both in the United States and in other countries.

Mention should also be made of the condition of fetal athyreosis in pigs, which Smith<sup>20</sup> showed to be due to iodine deficiency. In the western United States alone, many thousand pigs have been saved by the addition of iodine to the feed.

### Balance Studies and Requirements

Balance studies on man are not numerous but data available indicate that the iodine requirements are very small. In 1923 McClendon and Hathaway<sup>21</sup> reported the results of a balance experiment on a young



man aged 23. The average intake during a three-day period amounted to 57  $\mu$ g. of iodine daily; the total iodine excreted in this period was 21  $\mu$ g./day. This subject was therefore in positive iodine balance under the conditions of the experiment, even though he was a resident of the goiter belt.

Fellenberg<sup>6</sup> has undertaken elaborate studies of his own iodine balance. He determined, by analysis, the intake of iodine in the food, estimated the retention of iodine from the air, and measured the outputs in the urine, feces, sweat and nasal excretions. Studies were made during periods of starvation, and during periods of low- and various high-iodine intakes. When the total amount of iodine ingested amounted to 14  $\mu$ g./day over a period of 15 days, 13.1  $\mu$ g./day were excreted. Hence, the subject was in slightly positive iodine balance. Further experiments showed that a greater positive balance resulted from increasing the iodine intake by ingestion of potassium iodide or foods which are relatively rich in iodine, such as water cress, sardines or cod-liver oil.

Cole and Curtis<sup>8</sup> found that four adults remained in equilibrium or in positive balance when the daily intake of iodine in food and water amounted to 39-162  $\mu$ g./day. Another subject was in negative balance when the intake was 158  $\mu$ g./day. Obviously short-time balance studies do not take into consideration the reserve stores of the body. The experiments of Cole and Curtis show, however, that iodine equilibrium can be maintained in an adult if the intake is of the order of magnitude of about 0.1 mg./day (100  $\mu$ g.).

Scheffer<sup>29</sup> reported additional balance studies which likewise show that equilibrium in a normal subject can be maintained on intakes of 54-155  $\mu$ g. daily. In his subjects most of the iodine was excreted by way of the urine, only small amounts appearing in the stools; but in some cases excretion by the skin amounted to more than the combined excretions in urine and feces. Some of the Scheffer experiments indicate that persons with Basedow's disease or thyreotoxicosis did not maintain equilibrium on "normal" intakes. Some of these patients lost considerable amounts of iodine through the skin.

Fellenberg has reported the results of some metabolism studies on an eight-year old girl. Prior to the investigation this subject had been receiving iodine therapy, but no extra iodine was given during the six weeks immediately before the experiments were begun. On an intake of 38  $\mu$ g. of iodine daily this child was in negative balance. When small amounts of potassium iodide were added so as to increase the iodine intake, the balance became positive.

Data from balance studies are too few to warrant any precise formulation of iodine requirements. The evidence does show that the normal requirements must be small, possibly 0.05 or 0.10 mg. daily. Additional evidence shows that these small requirements may not be met by foods

and drinking water. The usual intakes of iodine are most likely to be deficient during growth, particularly during adolescence, and in pregnant and lactating women. There is evidence also that the iodine demands are increased during fever and infections.

### Sources of Iodine

Ordinarily we receive our iodine from foods and drinking water. Available evidence indicates that the amount of iodine in these sources is extremely variable. In the absence of iodine fortification of foods or drinking water, or the administration of iodine-rich fertilizers to the soil, the chief determinant of iodine intake is geographical situation.

It has been stressed by Fellenberg and others that iodine is widely distributed in soils and water supplies but it never occurs in very large amounts. The richest source of iodine known is Chilean saltpeter which contains only 0.2 per cent of iodine. The iodine content of other mineral deposits is small but is uniformly present. This appears to be the principal determinant of the iodine content of the soil. Iodides in general are soluble salts and tend to be leached out by rain water. The presence of organic matter in soil helps to retain the iodine content. The iodine content of plants used as foods depends largely on the iodine of the soil in which they are grown.

Potable waters usually contain iodides in small amounts, 0.2-2  $\mu\text{g./l.}$  or more. Less than 0.2  $\mu\text{g./l.}$  is usually found in those regions where goiter is endemic. This does not usually mean that the amount of iodine in the water determines the incidence of goiter, but it does reflect the iodine content of the soil and hence of the vegetables grown in the soil. As McClendon<sup>20</sup> has shown, some mineral waters are comparatively rich in iodine. Water from a deep well in Texas contained 185  $\mu\text{g.}$  of iodine per liter.

Orr and Leitch<sup>23</sup> have described some interesting observations in the nineteenth century by Chatin, concerning the drinking water of two small villages on the banks of the Rhone. In one of these towns goiter was universal and cretinism common; in the other these conditions were not observed. Later the water supply of the goiter-free village was changed to avoid the outflow of a hot spring. Thereafter goiter and cretinism became common in this village. Chatin analyzed the water and showed that the water from the hot spring contained considerable iodine while the supposedly more desirable drinking supply was markedly low in this element.

The iodine content of plant foods is extremely variable. In general, garden vegetables and legumes contain more iodine than cereals and fruits. Water plants contain more iodine than land plants. Certain sea weeds such as kelp, ordinarily not considered as desirable foods for man, are relatively so rich in iodine that their indiscriminate use is ill-

advised. Sea foods such as lobsters, sardines, other fish, etc., are comparatively rich in iodine. Milk contains small amounts of iodine and the amount present can be increased by administration of iodides to the cow. Orr and Leitch have reported that the iodine content of cow's milk in a non-goitrous district ranged from 4 to 7  $\mu\text{g.}/100\text{ cc.}$  When small amounts of iodine were added to the feed, the iodine content of the milk could be increased to more than 30  $\mu\text{g.}/100\text{ cc.}$  In goitrous regions, the iodine content of cow's milk may be so small as to be scarcely detectable. It has been shown that the iodine content of eggs, particularly the yolks, can be increased by feeding iodides to the hens.

**Iodine supplements.**—The iodine content of foods and drinking water is so variable and usually so small that various methods of increasing the iodine intake have been suggested as a means of prophylaxis against simple or endemic goiter. The methods which have been tried on a large scale are the addition of iodine to water supplies, the administration of iodides or tablets containing iodine at regular intervals, and the use of iodized salt. Any of these methods is effective for the people whom it reaches. The addition of iodine to water supplies does not affect the rural population. The administration of iodine preparations to school children does not reach the adults. Probably the addition of iodine to table salt benefits the most people, although the amount of salt used by different persons varies widely.

In the United States the proportion of either sodium or potassium iodide to salt in iodized salt is commonly 1/5000. This is considerably higher than the ratios which have been adopted in some other countries.<sup>2</sup> In Switzerland for example iodized salt is dispensed which contains 1/200,000, and in New Zealand iodized salt has been introduced which contains 1/250,000.

Some persons, fortunately very few, are hypersensitive to iodine. It has not been shown that iodized salts harm these individuals, but medicinal quantities of iodides are not well tolerated. There is evidence that the administration of iodides to persons with the adenomatous type of goiter is harmful.<sup>14</sup> The unwise use of iodides by these individuals may cause the development of toxic goiter, although surveys have shown that iodized salt has not been responsible for any harmful effects.<sup>22</sup> The value of iodized salt in the prophylaxis of simple goiter is established.<sup>2, 7, 11, 16, 26, 27</sup> The possibility remains, however, that harm may result to some persons. For these reasons the Council on Foods of the American Medical Association,<sup>4</sup> in accepting iodized salt, cautions that every person over thirty who has a swelling on the neck should consult a competent physician before taking iodized salt or any other form of iodine therapy.

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## Chapter 11

### Traces

The importance of inorganic elements which occur in small amounts or traces has only begun to be appreciated, and no present account can hope to be adequate, but merely to indicate the development in this field.

The traces of most interest in physiology include aluminum, arsenic, bromine, cobalt, copper, fluorine, manganese, nickel, silicon and zinc. Iron and iodine, which are of first importance, are in a group by themselves, and are discussed in separate chapters. Of less importance are lithium, rubidium and caesium, which may accompany other alkalis. Klinke<sup>8</sup> has treated these elements according to their grouping in the periodic system, but no relationship in the same group can be deduced (as for example between fluorine, chlorine, bromine and iodine), much less between groups.

The discovery by Baumann of iodine in the thyroid gland brought realization of the fact that materials present in amounts so small as to have been thought negligible might nevertheless be essential to vital processes. If the thyroid required iodine, might not the other glands of internal secretion require other mineral traces?

It has long been known that iron found in the protein, hemoglobin, is essential for respiration. The discovery of copper in hemocyanin of crustaceans,<sup>5</sup> of zinc in sycotopin of oysters<sup>199</sup> and of vanadium in the blood pigment of sea squirts<sup>7</sup> again disclosed that traces of minerals might exert profound and specialized physiological effects.

With the advent of the vitamin era there was a twofold stimulus to the study of traces. First, the dramatic effect of minute amounts of organic material on nutritional success or failure left nothing too small to be searched for. The second stimulus, rising from the first, came from the necessity for feeding purified diets.

Osborne and Mendel, in 1913, attempted to find a salt mixture which would supply the essential minerals. In 1918 they reported their successful salt mixture IV, which was made in imitation of the minerals in cow's milk. Manganese, aluminum and fluorine were included as traces. With this mineral addition they were remarkably successful. McCollum, however, secured successful nutrition without the inclusion of these traces.

There is a question whether the purified diets used still contained enough impurities to supplement the known ingredients.

The widespread occurrence of the "trace" elements in plants and animals has long been known. Albu and Neuberg<sup>1</sup> prophesied that plants would be found to contain all the elements of the soil in which they grow. This has been substantiated; and conversely, if the soil does not contain the traces, both the plants and the animals eating them may suffer from the deficiency. The analysis of mineral waters given by Albu and Neuberg<sup>1</sup> includes traces, presumably on the basis that herein might lie the efficacy claimed for them. Arsenic, chromium and vanadium have been found in the soil, and the last in toxic wheat, which also contains selenium.<sup>4</sup> Molybdenum and tellurium have been found in native plants.<sup>2, 3, 4</sup> The last two elements will not be discussed further.

From the point of view of nutrition with mixed diets the intake of traces is adequate except in very special circumstances. The two outstanding cases are iodine deficiency in low-iodine areas, and iron deficiency in milk diets. On the other hand, natural foods and water supplies may contain enough of certain traces, such as selenium or fluorine, to be harmful.

The occurrence of many trace elements in animal tissues may be exemplified by milk, which has been shown to contain boron, fluorine, lithium, manganese, rubidium, silicon, strontium, titanium, vanadium and zinc.<sup>14</sup> Nearly every element has been reported found by spectroscopic methods in living tissue.

From the list of those cited, the probability is that aluminum, arsenic, chromium, caesium, lithium, molybdenum, rubidium, strontium, tellurium, titanium and vanadium are not essential for nutrition. Those of known importance are bromine, copper, manganese and silicon. Those of doubtful value are cobalt, fluorine, nickel and zinc.

Brief reviews of some or all have been given by Klinke,<sup>8</sup> The White House Conference,<sup>13</sup> Sherman,<sup>11</sup> Rose<sup>9, 10</sup> and Hart and Elvehjem.<sup>6</sup> Spiro<sup>12</sup> has reviewed the pharmacology of the "trace" elements.

### Aluminum

Because this element occurs widely in plants, it forms part of the dietary of all animals.<sup>20, 21, 26</sup> Except in the case of *lycopodium*, of which aluminum may constitute 57 per cent of the ash,<sup>1</sup> it is present in only minute amounts. It is probably less than one-half part per million of either plant or animal matter, as determined by means of the quartz prism spectograph.<sup>25</sup> Many other investigators report aluminum in tissues in varying quantities.<sup>19, 27, 29</sup> The amount in any event is extremely small; most was found in brain—about 2  $\mu\text{g./gm.}$

Considerable interest has been aroused by the problem of ingestion of aluminum other than that contained in ordinary foods, namely,

aluminum in food contaminated by corrosion of aluminum cooking utensils,<sup>15, 16, 23</sup> and that in alum baking powders.<sup>28</sup> The answer has become one of theoretical interest only, for it has been shown that aluminum is resistant to corrosion, and that considerable amounts of ingested aluminum exert no toxic effect. McCollum *et al.*<sup>25</sup> found no difference in growth or reproduction of rats fed an aluminum-free diet, or one containing 0.6 per cent of  $\text{AlCl}_3$ , or the equivalent in baking powder.

An American diet was found to contain over 3.0 gm. of aluminum per week. Practically all ingested aluminum is excreted in the feces. In large amounts it may interfere with the absorption of phosphorus, and cause low serum phosphate.<sup>17, 18, 22, 24</sup>

A review of the literature to 1927 is given by Rose.<sup>9</sup>

### Arsenic

It was shown by Gautier in 1899<sup>36</sup> that arsenic is a normal constituent of living matter, and this was confirmed by Bertrand.<sup>31</sup> It may occur in organic combinations which the mould *penicillium brevicauli* makes volatile. It is widely distributed in human tissues in a concentration of about 0.1 mg./100 gm.<sup>32, 37</sup> It accumulates in the hair and nails.

No one has shown arsenic to be an essential in nutrition. It is present in the diet, especially in sea foods.<sup>33, 38</sup> The urine may contain 0.5 mg./day,<sup>30</sup> but the major part of the excretion is by way of the intestine.<sup>34</sup> An interesting metabolism study of the milch cow<sup>35</sup> shows that arsenic normally is not excreted in the milk, but such is not the case when toxic doses are given. When 0.287 gm. was given there was storage in every case. By slowly increasing the dose, a tolerance against arsenic may be developed. The Styrians are said thus to have increased their weight, strength and clear complexions. The therapeutic use of arsenic extends back to the Middle Ages. It has been widely used as a tonic for anemia, and for various skin disorders, and since Ehrlich, for syphilis and sleeping sickness. Overdoses cause gastro-intestinal symptoms and liver destruction. More will undoubtedly be written of its biologic role.

### Bromine

Bromine occurs as a normal constituent of all tissues. In spite of its well-known pharmacological properties, little attention has been paid to its physiological importance until recently. Its concentration in the hypophysis is such as to warrant further investigations, and it is not beyond the realm of possibility that it should some day occupy a position comparable to its better known sister, iodine. The bromine content of most organs is about 1 mg. per cent,<sup>40, 41</sup> but in the hypophysis it is 15-30 mg. per cent—highest in the anterior lobe, next in pars intermedia, and only in small quantities in the posterior lobe (cattle, dogs

and human beings).<sup>55</sup> Studies were made of various sections of brain during sleep, and a lowered bromine content of the hypophysis was found (not true with narcotics).

The bromine content of plants ranges from 0.17 to 2.02 mg./100 gm. of dry matter.<sup>42</sup> Watermelons and tomatoes supply the greatest amount.

Although bromine is similar in metabolism to chlorine the two are not interchangeable. Given in large amounts it produces stupor and a skin eruption. Injected bromine is distributed throughout the body and is in part excreted with the gastric juice.<sup>49</sup> Like chlorine, ingested bromine leaves the body by way of the kidneys. But the kidneys have a greater facility for the excretion of chlorine than bromine, and ingestion of bromides in excess leads to generalized edema.<sup>45, 48</sup>

The bromine concentration in serum averages 1.0 mg./100 cc. and of cells about 0.5.<sup>44</sup> The bromide distribution ratio between the cells and serum was found to be 10 per cent higher than the chloride, and to follow the Donnan equilibrium *in vitro*, but not *in vivo* after ingestion.<sup>46, 52</sup> The [Br<sup>-</sup>] to [Cl<sup>-</sup>] ratio in cerebrospinal fluid was found to be the same as in plasma.<sup>43, 50</sup> The [Br<sup>-</sup>] of whole blood<sup>47</sup> is lowered to approximately half the normal value in manic depressive psychoses.<sup>54</sup> Change in the bromine distribution in blood was caused by administration of thyroid, and of extract of posterior lobe of the pituitary.<sup>51</sup> The [Br<sup>-</sup>] to [I<sup>-</sup>] ratio has been found constant.<sup>39</sup>

A synthetic diet containing less than 0.5 mg. of bromine per kg. permits growth of rats from weaning to maturity. Such animals have a very low bromine content and produce young low in bromine. The young do not survive. However, addition of bromine to the diet did not result in viable offspring.<sup>53</sup> The question of the necessity of bromine must therefore be deferred until the effects on the second generation can be studied.

### Cobalt and Nickel

Bertrand and Mâcheboeuf<sup>56, 57</sup> were largely responsible for the interest in these elements. The presence of cobalt and nickel in the pancreas led to the suggestion that there is a relation between them and insulin. However this has not been substantiated.<sup>58</sup>

The addition of cobalt to a normal diet produces polycythemia in rats; the cells are increased from ten million to nearly fifteen million per cmm.<sup>61, 62, 63, 65</sup> When milk diets are supplemented by iron or iron and cobalt, anemia is produced, or if anemia is present it is not cured. The production of red cells in excess of normal is found when iron, copper and cobalt are added. Further addition of manganese enhances this effect.

Ingested nickel and cobalt are excreted principally in the feces,<sup>60</sup>



but a slightly greater percentage of cobalt than nickel is found in the urine.<sup>59</sup>

The whole rat contains less than 0.01 mg. of cobalt. When fed 0.5 mg. daily for weeks the content may be increased to 0.05-0.07 mg.<sup>64</sup>

The most recent reports indicate that cobalt must now be considered a dietary essential.\*

## Copper

Until recently the interest in the physiology of copper was restricted to the marine bluebloods—the gastropods and arthropods. The importance of the discovery of copper in octopus blood in 1847<sup>5</sup> and isolation of hemocyanin by Fredericq in 1878 have been reviewed by Severy,<sup>112</sup> and Vickery and Osborne.<sup>116</sup> Recent studies have shown this to be a protein, similar to hemoglobin, in which the copper acts to transport the oxygen.<sup>101, 102, 103</sup> Hemocyanin thus represents the bronze age of living matter, and hemoglobin the iron age. Neither the form in which copper is present in mammals, nor its mode of action is known. Copper salts are active in biological oxidation-reduction systems,<sup>94</sup> and probably catalyze many reactions (see Chapter 8, *Sulfur*).

Table 21.—Distribution of Copper in Tissues of Adult Rats.\*

Tissue	Moisture (%)		Dry Weight (gm.)		Copper Absolute (mg.)		Copper in Dry Matter (mg./kg.)	
	A	B	A	B	A	B	A	B
Bone .....	30.6	31.2	40.15	38.78	0.1004	0.1648	2.50	4.25
Brain .....	76.2	76.1	0.41	0.42	0.0037	0.0047	9.14	11.16
Heart .....	80.6	75.9	0.25	0.28	0.0025	0.0034	9.92	12.25
Kidney ....	74.4	72.9	0.92	0.93	0.0140	0.0254	12.41	27.20
Liver .....	72.6	74.2	5.11	4.50	0.0584	0.9600	11.43	213.32
Lung .....	77.0	78.6	0.81	0.67	0.0050	0.0071	6.21	10.67
Muscle ....	70.3	69.0	46.80	44.56	0.0936	0.0936	2.00	2.10
Skin .....	51.7	58.5	45.20	39.22	0.1614	0.1569	3.57	4.00
Spleen .....	75.6	70.4	0.29	0.28	0.0010	0.0049	3.41	17.47
Testicle ...	85.9	85.7	0.55	0.50	0.0048	0.0050	8.65	10.00
Total ...			140.48	130.14	0.4422	1.4258	3.15	10.96

\*Lindow, Peterson and Steenbock.<sup>57</sup> Reproduced by permission of the *Journal of Biological Chemistry*.

A = Animals fed stock diet. Age of rats, 7-8 months.

B = Animals fed stock diet + 5 mg. copper per day for the last 46 days.

**Occurrence of copper in the body.**—The total amount of copper in the human body has not been determined, but it occurs in all tissues—a fact which did not escape Thudichum. Data for copper in the whole body as well as in the separate organs of rats fed varying amounts of copper are given in Table 21. The largest amounts of copper are found

\* *J. Nutrition*, 15, Suppl., 22 (1938).

in the liver, spleen and kidneys.<sup>77, 99</sup> The copper content of the human brain is 3.0-6.0 mg./kg.<sup>69</sup> Human blood contains 1.0-2.0 mg./l. of serum.<sup>79, 80, 85, 109, 115</sup> Most of the copper is in the corpuscles.<sup>74, 81</sup> Human milk contains 0.4-0.6 mg./l., a value higher than that of cow's milk.<sup>79, 83, 119</sup> Copper has been found in gall stones to the extent of 3.0 gm./kg.<sup>93, 111</sup>

**Role of copper in anemia.**—The pernicious anemia problem is not directly related to copper metabolism. The secondary anemia problem studied by Whipple and associates has been reviewed by Robschey-Robbins.<sup>106</sup> In their studies of the cure of anemia from hemorrhage they found that plants or the ash of plants had a greater effect than could be attributed to iron alone, and thus opened the question of the value of traces. (See also Chapter 9, *Iron*.)

Nutritional anemia develops in both infants and experimental animals maintained on an exclusive milk diet. It was believed that the deficiency lay entirely in the lack of sufficient iron in the milk until the dramatic statement by Hart, Steenbock, Waddell and Elvehjem<sup>82</sup> that copper is necessary together with iron. After about four to six weeks on an exclusive milk diet young rats cease to grow, the hemoglobin concentration and the red blood cell count show a condition of severe anemia; the animals are weak and, unless treated, soon die. If solutions of pure inorganic iron salts are added to the milk, even from the beginning of the milk feeding, the end results are not essentially different. But if the animals, even when severely anemic, are given iron and also a small amount of a copper salt, there is a vast difference in their subsequent behavior. Within a day or two they become more alert, and eagerly consume their food and the dietary supplements. The red cell count, initially 2-4 million per cmm. of blood, ultimately attains the level of 8-10 million per cmm., which is normal for the rat. The hemoglobin also increases from the extremely anemic levels of about 2 gm. of hemoglobin per 100 cc. to 11-15 gm./100 cc. Complete recovery requires one to four weeks, or longer, depending largely upon the dosage of iron, and occurs only if copper is available to the animals.<sup>82, 92, 98</sup>

There have been some reports that, although traces of copper accelerate formation of hemoglobin, copper is not essential, and that salts of other metals can produce the same effect.<sup>86, 96</sup> It is now generally believed, however, that copper is essential and its action specific.<sup>97</sup> It has already been stated that traces of cobalt, in conjunction with iron and copper, produce a polycythemia.<sup>61</sup> The polycythemia of low-salt diets is associated with low hemoglobin concentrations and has been studied by Smith and Schultz.<sup>114</sup> A similar condition has been produced in rats fed a milk diet, by administration of adequate amounts of copper but deficient amounts of iron.<sup>76</sup> Manganese plays no part in hemoglobin production, but on a milk-iron-copper diet, the addition of man-

ganese improves the rate of growth and permits the animals to reproduce.<sup>115</sup>

It has been shown that the anemic rat may secure and utilize copper from various sources. In the early experiments of the Wisconsin investigators, it was observed that the addition of plant materials would permit recovery from nutritional anemia of rabbits fed on milk and salts of iron. The ashes of the plant materials were also effective; subsequent investigation showed the presence of copper in the ash. Impure salts of iron, which later were found to contain traces of copper, were curative and carefully purified iron salts were not.<sup>117</sup> As secreted by the cow, milk contains from about 0.10 to 0.20 mg. of copper per liter.<sup>75, 79, 100, 119</sup> When rats are fed on milk which contains these small amounts of copper, supplying about 2  $\mu$ g. of copper daily, they fail to show a response in hemoglobin production when salts of pure iron are administered by mouth.<sup>98</sup> Slow increases of hemoglobin are observed when the copper intake is increased to about 8  $\mu$ g./day. If the milk contains 0.35 mg./l. of copper, the intake of this element is sufficient to permit a slow but constant increase of hemoglobin when supplements of pure iron salts alone are administered. The copper content of milk obtainable on the market may be 0.35 mg./l. or even higher.

Convincing evidence of the importance of copper is afforded by further observations. There is a condition of anemia which develops in cattle which have grazed on pastures demonstrably low in copper.<sup>67</sup> This anemia cannot be cured by iron salts, but administration of salts of copper brings about improvement, thus showing the existence of a copper deficiency. When testing the value of egg-yolk, known to be an excellent source of iron, with anemic rats, Sherman, Elvehjem and Hart found at first that it was not utilizable. Further studies demonstrated that the iron of egg-yolk is as available as the iron in an equivalent amount of ferric chloride, but the copper of the yolk is in a form which cannot serve as a source of the element to the body.<sup>113</sup>

Although other substances, particularly amino-acids, are necessary in the actual production of hemoglobin, these are supplied in adequate amounts by the milk. Glutamic acid, salts of arsenic, mercury, etc., and vitamin D (in the form of viosterol) do not cure anemia.

Anemic rats, which die when administered salts of iron alone by mouth, are cured when the iron is injected intraperitoneally.<sup>84a</sup> There is a simultaneous increase of the amount of copper in the tissues of the injected rats.<sup>68</sup> This increase in copper content has been attributed to a greater utilization of the copper of the milk. The exact relation of iron and copper in metabolism is not understood, but it is apparent from such observations that these two elements are intimately associated in hemoglobin production.

The mechanism of the hematopoietic function of copper is under

investigation. The liver of the fetus acts as a storage depot for copper.<sup>107</sup> Cunningham<sup>70</sup> and also Elvehjem<sup>71</sup> have shown that iron may be stored in the liver without increase of hemoglobin. When copper is given, this stored iron is utilized to form blood; not only is the hemoglobin increased, but a rapid increase in cells occurs. These appear first as small reticulated cells, poor in hemoglobin, which gradually become normal with recovery.

**Copper content of foods.**—The copper content of a large number of foodstuffs has been given.<sup>86, 90, 91</sup> Table 22 shows the order of magni-

Table 22.—Degree of Variation in Iron, Manganese, and Copper Content of Different Classes of Food Materials.\*

Class	No. of Samples	Average (mg./kg.)	Minimum (mg./kg.)	Maximum (mg./kg.)		
Iron in Fresh Material						
Fresh fruits	23	6.6	2.3	Watermelon	22.8	Grapes
Nuts	12	41.0	21.4	Walnut	79.2	Pistachio nut
Roots and tubers	14	11.0	3.0	Onion	23.6	Beets
Vegetables, leafy	7	69.0	3.4	Cabbage	192.1	Parsley
Manganese in Fresh Material						
Fresh fruits	13	4.0	0.2	Watermelon	22.9	Blueberries
Nuts	3	13.3	6.3	Pistachio	18.0	Walnut
Roots and tubers	7	3.2	0.5	Onion	13.5	Beets
Vegetables, leafy	8	6.6	0.8	Cabbage	12.6	Beet greens, tops
Copper in Fresh Material						
Fresh fruits	27	1.0	0.2	Strawberries	3.4	Olives
Nuts	10	11.6	6.0	Chestnuts	14.3	Hickory nuts
Roots and tubers	11	1.4	0.8	Carrots	2.7	Oyster plant
Vegetables, leafy	14	1.2	0.4	Watercress	3.1	Artichoke

\* Lindow, Elvehjem and Peterson,<sup>86</sup> Table 2. Reproduced by permission of the *Journal of Biological Chemistry*.

tude as well as the wide variations which occur. Calf's liver contains as much as 44.1 mg./kg.; beef liver, 21.5; oysters, 30.7;\* fresh fish, about 2.5; meat, 1.0; vegetables, 1-3. Remington and Shiver<sup>104</sup> point out that the leaves contain more copper (also iron, manganese and iodine) than do the roots of the same plant. Copper in plants varies with that in the soil.<sup>72, 95</sup> Feeding of copper does not alter the concentration in milk<sup>75</sup> or in eggs.<sup>73</sup>

In France, copper has sometimes been used to make canned vegetables green. Cooking in copper vessels also increases the color of peas and string beans, but the vitamin C is diminished thereby.<sup>84, 105</sup>

Some copper is undoubtedly absorbed, as it is found in urine of infants, children and adults—0.02-0.10 mg./day.<sup>83</sup> Flinn and Inouye<sup>78</sup>

\* The copper of the oyster has been found to be greatly increased when these grow near copper mines in England, but the green color of those from the oyster beds of Marie Antoinette is due not to copper, but to green algae.

fed rats 2 mg./day for a year without harmful effects, although hemochromatosis has been reputed to follow high copper intake.<sup>88, 89</sup> It may be of importance in the cause of cirrhosis of the liver.<sup>108, 110</sup> Improved growth has been associated with adequate copper intake. The average intake of human beings has been estimated by Cunningham<sup>70</sup> to be five times the requirement.

### Fluorine

As early as 1803, over 80 years before the element was isolated, Morichini found fluoride in the bones of a fossil elephant. This was confirmed by Gay-Lussac and Berzelius. Bones, and especially fossil bones and teeth, contain the greatest amount of fluorine in physiological material. The earlier literature was reviewed by Gabriel<sup>127</sup> in his effort to find the part played by fluorine in the composition of normal bone. Different authors and methods give variable data. The values range from 0.1 to 0.3 per cent of bone ash, and for the teeth of elephants and mastodons, from 2.5 to 3.0 per cent.<sup>123</sup> The enamel is especially rich in fluorine; it contains approximately twice as much as bone. In oyster shells the fluorine content is ten times that of sea water. All tissues contain fluorine. The fluorine concentration of blood is 0.5 mg./100 cc.;<sup>128, 143</sup> this is increased in hemophilia.<sup>139</sup>

Fluorine is present in practically all foods and most mineral waters. Gautier and Clausmann<sup>129</sup> published analyses of 63 vegetables and found variations of 0.0006-0.138 per cent, the average being 0.026 per cent. The fluorine content of both cow's and woman's milk is 0.1-0.2 mg./100 gm. of fresh milk.<sup>128</sup> The fluorine content of milk is not increased by ingestion of fluorine, but that of eggs is.<sup>6</sup>

Bunge suggested that milk "might yet be useless for the growth of the human infant for want of traces of fluorine." Mazé<sup>130</sup> has found the content inadequate for growth and reproduction in rats, but Daniels *et al.*<sup>13</sup> attained success when only iron, copper and iodine were added. Fluorine, then, has never been demonstrated to be essential by biological experiments, although it may be when suitable experiments are designed.<sup>135</sup>

Excess fluorine has been shown to produce acute or chronic poisoning.<sup>126, 131</sup> The toxic effects of fluorine and hydrofluoric acid on the mucous membranes and respiratory passages are well known. Doses of 1.5-15 mg./100 gm. of body weight fed daily to rats caused stunting or death in a few weeks.<sup>137</sup> In chronic poisoning the fluorine accumulates not only in the bones, but also in tissues.<sup>141</sup> In acute poisoning the serum calcium may be markedly lowered. Injection of 10 mg./100 gm. is fatal to rabbits.<sup>121</sup> When soluble fluorides are fed, the bones and teeth retain the fluoride. The fluorine in bones of dogs increased on fluoride feeding from 0.03-1.7 per cent of the dry material.<sup>138</sup> Brandl

and Tappeiner<sup>122</sup> fed a 12.75-kg. dog 402.9 gm. of fluoride in 647 days; 330.5 gm. were recovered in the urine and feces. Over 64 gm. were found in the body, of which 60 gm. were in the skeleton. The dry skeleton contained 5.19 per cent of sodium fluoride. The amount present is proportional to the amount ingested. The bones of animals with fluorosis contain over one per cent of fluorine. The bone ash may be normal in amount with small intakes of fluorine<sup>140</sup> or increased with larger amounts; but the ratio of Ca/P is lowered,<sup>132</sup> which means that the bone calcium is diminished. About one-fourth of the excretion is found in the urine and the rest in the feces.

Fluorine in quantities of 0.03 per cent or more of the diet was found not only to retard growth but also to lower calcium balances. The phosphatase is diminished in fluorosis. It has been claimed and denied that fluorosis produces enlargement of the thyroid gland.

In 1925 McCollum and associates<sup>133</sup> showed that very small amounts of fluoride (226 mg./kg., or 0.02 per cent of fluorine), added to the diets of young rats, caused a chronic poisoning which markedly affected the teeth. Two mg. of fluorine daily for 3 months or longer caused the upper incisor teeth to overgrow so that they curved and pierced the palate, and the lower incisors were eroded to the gum line. The teeth were brittle, and became opaque and white. These findings have been amply corroborated not only for rats, but also for cattle, swine and sheep.<sup>120, 124, 140, 142</sup> A mineral supplement of rock phosphate (which contains 3-4 per cent of fluorine) will consistently alter the size, shape and texture of bones and teeth of animals. The apatite structure remains unaffected.<sup>134</sup>

The condition of fluorosis in teeth occurs not only in experimental animals but also in people. Here it is known as mottled enamel. It was first described in 1901 by Sager, although its relationship to fluorine was not demonstrated until many years later. Mottled enamel is known to occur all over the world. In the United States Dean<sup>125</sup> has studied over 300 areas in which it is endemic. More than one-fourth of these are in Texas. Mottled enamel occurs especially in the permanent teeth of children exposed for more than the first eight years of life. First the teeth become chalky white and then turn brown. The occurrence is definitely correlated with the amount of fluorine in the drinking water. It is present when the water contains 0.9 mg./l. or more. At 2.5 mg./l. the incidence is 75-80 per cent, and at 6 mg./l. practically all show the condition.<sup>125, 136</sup>

The literature on fluorine has been reviewed by McClure<sup>131</sup> as well as in the general articles on traces.

### Manganese

Without manganese simple plants cannot grow, and complex ones become etiolated. They store manganese in leaves and seeds.<sup>144, 149, 157</sup>

Orent and McCollum<sup>161</sup> have convincingly demonstrated that manganese is an essential element for animals also. McHargue<sup>90, 158</sup> and McCarrison<sup>156</sup> feel that the role of manganese in nutrition is of great importance.

Manganese occurs in all human tissues. The blood contains 2  $\mu\text{g.}/100$  cc., about twice as much in plasma as in cells, and the concentration is not increased in manganese poisoning. The liver contains the largest amount of manganese, 170  $\mu\text{g.}/100$  gm. of fresh tissue, the kidney 61, the lungs 20<sup>164</sup> and nervous tissue still less. The colon contains more than the upper bowel, which indicates that this is the probable site of excretion. The values are higher in birds than in mammals, but of the same order of distribution. The greatest concentration has been reported in crawfish, 10 mg./100 gm. of fresh tissue.

On a manganese-free diet male rats showed testicular degeneration. The females when mated with normal males produced young. They were unable to nurse such young, and foster mothers also refused to rear 100 out of 107 of them.<sup>161</sup> Daniels and Everson<sup>146</sup> have shown that the main defect is in the young, which contain 65 per cent less manganese than normals. The mothers on low-manganese diets were able to nurse normal young successfully. Additions of 0.005 per cent of manganese rendered the diet normal. Orent and McCollum<sup>161</sup> suggest that the function of manganese may be primarily with the development of the anterior pituitary and its regulation of the sex glands. The claim by the Wisconsin group that it is necessary for normal oestrus cycle has not been confirmed.<sup>162</sup>

Manganese has been used clinically for generations in the treatment of secondary anemia. The question of whether manganese could serve as an activator for the utilization of iron in milk anemia led to positive findings,<sup>148, 159</sup> but these have been contraverted by the Wisconsin workers<sup>168, 171</sup> and others.<sup>96, 115, 151, 154</sup>

The Wisconsin group have studied manganese in the body and also its metabolism.<sup>152, 153, 168, 169</sup> Ingested or injected manganese rapidly leaves the blood. In patients with fistula the bile content was found to be increased. Ingested manganese was excreted almost wholly in the feces. It has been found that the addition of manganese to a milk diet supplemented with iron and copper doubled the rate of growth of mice. The effect on the growth of rats is more difficult to demonstrate because these animals may grow and reproduce without addition of manganese to the milk. When fed in considerable amounts manganese is stored, especially in the liver and secondarily in other glandular organs and less in bones and skin. Subcutaneously,  $\text{MnCl}_2$  is toxic, and this has been related to cirrhosis of the liver.<sup>145</sup> The oxides are less toxic.

Cow's milk contains 0.03 mg./l. regardless of whether the intake is 1 or 5 gm./day; goat's milk contains 0.08 mg./l.<sup>153</sup> Colostrum contains

five times as much as milk; this may be a protective factor.<sup>170</sup> The manganese content of plants and foods has been reviewed by recent workers.<sup>90, 91, 104, 150, 155, 162, 165, 166, 168</sup> According to the recorded values (see Table 22), blueberries are the most concentrated biological source of manganese. The bran of cereals is also especially rich; nuts come next; animal products, dairy products and fish contain less than 1 mg./kg. The manganese content of plants is increased by the addition of this element to the soil.<sup>95</sup>

The manganese requirements of man are not known. Everson and Daniels<sup>147</sup> suggest that the diet of children should contain between 0.2 and 0.3 mg./kg. of body weight per day. McCarrison<sup>156</sup> feels that milling of cereals has reduced the manganese in our diet below the safety point, especially for the needs of the thyroid gland. He thinks that children should take whole-wheat bread because of its manganese content alone.

The literature on manganese in physiology has been reviewed by Reiman and Minot,<sup>164</sup> Rose,<sup>9, 10</sup> the White House Conference,<sup>13</sup> von Oettingen<sup>160</sup> and Hart and Elvehjem.<sup>6</sup> The whole subject is in a state of active expansion.

### Selenium

In the western part of the United States occurs a condition in cattle called alkali disease, or "blind staggers," according to whether it is chronic or acute.<sup>3, 172</sup> It has recently become evident that this is due to an excess of selenium in the food. Apparently the selenium is obtained from the vegetation which grows on soil containing 0.5-40.0 mg./kg. of selenium. Knight<sup>173</sup> has stated that the selenium problem involves no great menace to public health; so far it has not been reported as deleterious to people. The recent literature has been reviewed by Hart and Elvehjem.<sup>6</sup>

### Silicon

Silicon occurs in all plants, where presumably it plays an important part structurally and chemically. It is part of the intake of all animals, especially herbivora. The intake of human beings amounts to several hundred milligrams per day.<sup>177</sup>

Silicon is present in the new-born to the extent of 0.05 gm.  $\text{SiO}_2$ .<sup>170</sup> It occurs in all tissues, probably not as a contaminant, although analyses involve uncertainty due to its presence in reagents.<sup>14, 175, 178, 179, 182</sup> There are wide variations in the different organs; the kidney and pancreas contain most, and the brain least. It may equal 1-9 per cent of the ash. The silicon in skin may have to do with its elastic properties; it decreases with age.<sup>180</sup> Values for silicon in the blood as high as 30 mg./100 cc. have been given; recent determinations give 16 mg./100 cc. After injec-



tion this is increased, and may remain elevated for weeks.<sup>181</sup> Silicon dioxide is deposited in the lungs from dust, and produces a condition called silicosis. Normally the lungs contain 140 mg./100 gm. of dried material; this may be increased to 12.3 per cent in silicotic lungs.<sup>176, 179</sup>

Silicates undoubtedly are absorbed, and are excreted in part in the urine, though mainly in the feces.<sup>174, 183</sup> The amount in the urine of herbivora is sufficient to require computation in the anionogen-cationogen balance.<sup>35</sup>

## Zinc

Zinc occurs in all tissues and foods in amounts comparable to iron. It was found in food in 1877. The snail, *syctypus*, was found by Mendel and Bradley<sup>199</sup> to contain zinc in the liver and in the respiratory protein of the blood, hemosyctypin. The function of zinc in human nutrition is unknown.<sup>90, 112, 188</sup>

The zinc content of the human body is approximately 3.0 mg./100 gm., or a total of 2.2 gm. Lutz<sup>198</sup> found, per 100 gm. of tissue, 16 mg. in hair, 10 mg. in bone, and in agreement with earlier work<sup>200</sup> 5-14 mg. in liver and kidney, 4-5 mg. in muscle and spleen, and 1 mg. in brain.<sup>69</sup> The rat and cat contain approximately the same concentrations. The zinc concentration of the body and liver diminishes during the suckling period. The blood in man and various species contains 0.5-0.7 mg./100 cc. —about twice as much in cells as in plasma.<sup>189</sup> Milk contains 4 mg./l., and eggs about 1 mg. each. The content is not increased by feeding or disease. Zinc has been found in gallstones.<sup>111</sup> The zinc is present in a non-ionized form,<sup>191</sup> insoluble in water, but extracted by acids.

Ordinarily 15-20 mg. of zinc are consumed daily by an adult; of this 0.6 mg. is found in the urine, and nearly all the remainder in the feces. Only in tuberculosis and uremia was the urinary zinc found to be increased, and then rarely more than twofold.<sup>193</sup>

Contrary to previous opinion, the Harvard studies have shown that zinc is non-toxic,<sup>185, 190</sup> which has been confirmed.<sup>194</sup> Rats fed 2-38 mg., and dogs and cats as much as a gram daily for over six months showed no ill effects.

Attempts were made by Bertrand and Benzon<sup>186</sup> to feed zinc-free diets, but as the diets were lacking in vitamins and other essentials and the control animals died, it is impossible to evaluate the significance of the zinc additions. Later experiments with zinc-free diets plus vitamins by Bertrand and Bhattacharjee<sup>187</sup> led to death of mice; addition of zinc had beneficial effects. Hubbell and Mendel<sup>197</sup> were able to prepare complete diets which afforded mice less than 0.005 mg. of zinc per day. Even when fed this regimen, the mice grew and thrived. Slight lag in weight increase, and slight diminution of the zinc content of the body were observed. Whether such a diet is adequate for suc-

cessive generations was not ascertained. Recent work has shown that 22  $\mu$ g. of zinc per day caused a 50 per cent decrease in growth and delay in intestinal absorption. Because pituitary transplants caused amelioration of the condition it was suggested that zinc is necessary for the formation or utilization of one of the pituitary hormones.<sup>196, 201</sup> It has also been suggested that zinc acts as a catalyst of organic oxidations.

Zinc is probably an indispensable element like magnesium,<sup>158</sup> but also like magnesium, very special experiments are necessary to demonstrate its function. Even if zinc should prove an essential element there is no danger of a lack in the diet. Oysters are the foodstuff richest in zinc; they contain 40 mg./100 gm.<sup>91</sup> Fruits, vegetables, milk and tissues contain 2-5 mg./100 gm. Commercial casein may contain upwards of 30 mg./100 gm. The data on foods have been reviewed.<sup>192, 197, 198</sup> The sea, soil and spring waters contain zinc; as much as 50 mg./l. have been reported in natural waters. Although fish can live in these, and rats can safely drink water containing 1 gm./l., it has been recommended that drinking water should not exceed 5 mg./l.<sup>184</sup>

The work on zinc has been reviewed by Lutz,<sup>193</sup> Rose,<sup>9, 10</sup> Herkel,<sup>195</sup> and The White House Conference.<sup>13</sup>

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## Chapter 12

### Water Metabolism

#### OCCURRENCE OF WATER IN THE BODY

Water may well be regarded as the master component in the living body, not only because of its preponderance among the body constituents, but especially because of its role in body functions. All the chemical reactions of the body take place in a water medium in which the other substances are either dissolved or emulsified. Due largely to it the body fluids form the "milieu interieur" of living organisms. It has been discussed as an integral part of mineral metabolism throughout the book. This chapter adds little that is new, but summarizes the material and brings it into focus from a different point of view. Excellent reviews are available.<sup>2, 37, 58, 60, 63, 69, 73, 76, 77</sup>

**Functions and forms of water.**—Water serves many purposes in the body: it permits convection and circulation; it serves as a solvent; it has a high dielectric constant; by its heat capacity and evaporation it regulates temperature; it is a medium for the reactions of metabolism; in it ionization takes place; it acts as a lubricant for joints and muscles; it removes waste products from the body; and it serves as a building material for growth.

Water occurs probably as polymers, or aggregates, of two, three, or four molecules. In the body it is thought to be dihydrol.<sup>9</sup> Only as steam does it exist as H<sub>2</sub>O; ice is trihydrol. "Heavy water" has recently been described, but its physiological actions are not discussed in this review.

The amount of water absorbed by colloids of the body was formerly thought to be considerable. Such water is called "bound" because it does not affect the osmotic properties of the solutes. Hence divergence from the values calculated for total water has been used for its measurement, as determined by the freezing point, degree of swelling, vapor pressure and other properties of solutions. There is considerable dispute as to the fundamental theory involved. A review of this subject has been given by Gortner.<sup>36</sup> In some biological systems bound water may constitute over 50 per cent of the total water. The content in the colloids of the higher mammals is thought by Greenberg and Greenberg<sup>38</sup> to be negligible. Similarly, the bound water has been

found to be of small amount in blood serum.<sup>82</sup> The studies of Hill<sup>44</sup> on muscle indicate that bound water does not exceed 2-4 per cent of the total body water.<sup>43</sup> Other investigators have, however, reported larger amounts. From the view point of water exchanges in the body it may be regarded as a problem of the second order of magnitude.

In addition to the water contained in the body, the hydrogen and oxygen of constitution in protein, fat and carbohydrate are in part converted to water by combustion within the body. Such water in foods must be taken into account when considering the water intake. Whenever body tissue is catabolized, as for example in starvation, water is made available from the burning of tissue as well as from the body fluids.

Water is distributed in the body within the cells, in intercellular fluid, in the circulating fluids (blood, lymph and cerebrospinal fluid), and in secretions (saliva, gastric juice, pancreatic juice, bile and intestinal juices). The composition, amounts and interchanges of these fluids are discussed in Chapters 2 and 3. The importance of regarding minerals, not in relation to the total body or organs, but to the water in which they are dissolved, was shown on pages 42-45. This development has come only in the last few years, and the newer studies testify to its fruitfulness. For instance, computations of the chloride content of the body on the basis of water content (p. 42) lead to the important conclusion that the body fluids are constant in chloride concentration at all ages from the smallest embryo to the adult.

**Blood volume.**—The distribution of water in the body is not fixed and constant, and fluctuations may be exemplified by variations in blood volume. Strange as it may seem, there is no uniformity of opinion as to the magnitude of the blood volume even in health. The reason for this divergence is that different methods yield widely varying results and no method is without objections. A survey of these has been given by Erlanger.<sup>25</sup>

The blood volume is relatively low in obese persons and high in emaciated ones; values are more nearly proportional to surface area than to weight. By the vital red dye method Brown and Roth<sup>16</sup> found blood volumes of 2500-4000 cc./sq. m., or 75-120 cc./kg. of body weight. The plasma volumes were 1400-2500 cc./sq. m. By the carbon monoxide method Chang and Harrop<sup>20</sup> found, in 8 normal young men and women, blood volumes of 2000-2900 cc./sq. m., or 63-76 cc./kg. (average, 7 per cent of body weight). The latter values correspond to the data given in Table 4 (p. 19).

These values may be used to calculate the relation of the distribution of extra- and intracellular water of the blood to that of the whole body.

Total water in the body = 41 kg. (see Tables 1 and 3, pp. 16 and 18).  
 Intracellular water =  $0.70 \times 41 = 29$  kg. (see p. 45).  
 Extracellular water =  $0.30 \times 41 = 12$  kg.  
 Total blood in the body =  $0.07 \times 70 = 4.9$  kg.  
 Blood cells =  $0.40 \times 4.9 = 2.0$  kg.  
 Water in blood cells =  $0.65 \times 2.0 = 1.3$  kg.  
 Blood plasma =  $0.60 \times 4.9 = 2.9$  kg.  
 Water in blood plasma =  $0.92 \times 2.9 = 2.7$  kg.  
 Blood water/total water =  $4/41 = 10$  per cent.  
 Blood cell water/total intracellular water =  $1.3/29 = 4.5$  per cent.  
 Plasma water/total extracellular water =  $2.7/12 = 22.5$  per cent.

It can be seen that the water in blood plasma constitutes more than one-fifth of the total extracellular water in the body, while the blood cell water is only an insignificant fraction of the total cellular water. However, the actual amount of plasma water is only twice that in the blood cells, and hence the exchanges between cells and plasma may be of great significance.

The data on blood volume have been given graphically for all ages by Peters and Van Slyke.<sup>70</sup> They show a progressive increase with age when calculated per sq. m., and when calculated per kg. of body weight, they show an increase up to one year, decline to puberty and subsequent rise. Both physiological and pathological changes which tend toward water retention increase blood volume, and those which cause dehydration diminish it. These authors have discussed in detail the effect on blood volume of temperature changes, exercise, stasis and posture, as well as various diseases.

Diminution or increase in plasma volume is caused, in almost all cases, by a transudation of electrolytes and water so that the protein content rises or falls. In surgical shock due to hemorrhage or to severe injury, especially to the intestines, the blood pressure and circulatory blood volume are greatly reduced. The blood is withdrawn into the dilated abdominal blood vessels. These become more permeable and fluids are lost from the blood stream by transudation. In severe shock the proteins may also pass out of the blood stream. The condition is usually greatly improved when the blood volume is increased by transfusion or by the injection of colloidal solutions such as gum acacia, which increase the oncotic pressure.

#### WATER EXCRETION

Water is lost from the body through (1) kidneys, (2) intestine, (3) lungs, (4) skin, (5) tears, (6) oral and nasal secretions, (7) sexual secretions, (8) milk secretion, (9) vomiting and fistulae. Of these the nature of urinary and fecal excretion have been described in Chapter 3, as have also the chemical composition of sweat and its relation to the excretion of minerals by the other channels. Milk secretion forms a separate problem (see *Milk*, p. 71). Only the first four sources of water

loss will be discussed here, because the others are of importance only under very special circumstances. The phase which will be considered is the amount of water lost through the various channels.

The fractions of water excreted by the various organs do not vary greatly in relative amount with age. Normally about 60-70 per cent of the water is excreted by the kidneys, 2-6 per cent by the intestine, and 25-33 per cent by the lungs and skin.

**Kidneys.**—Under ordinary conditions an adult produces 1000-1500 cc. of urine per day, and infant 450-600 cc. Normally the amount of urine is directly proportional to the fluid intake and inversely proportional to that excreted by the skin and intestine. If the total solids which require excretion are great, larger amounts of urine are excreted, for the capacity of the kidney to concentrate materials is limited. This condition usually results in an increased consumption of water. Urine volumes in excess of 10 l./day have been reported. If the amount of water ingested is not sufficient, the kidneys still excrete the necessary volume of urine, and take the water from the other excretions or body fluids. When the amount of material to be excreted is small, as for example in Folin's study<sup>30</sup> of low-protein diets, the amount of urine may become quite small; in his study the volume of urine was only 385 cc. (see p. 200).

**Intestines.**—The feces normally contain 70-80 per cent of water, and therefore account for about 50-200 cc. of water daily, in the adult. In the infant 80-85 per cent of water is not unusual, and the water excretion by this means approximates 35-100 cc. The amount of water in the feces is increased with increased water intakes. Huge amounts of water may be lost by way of the bowel in diarrhea. Severe dehydration may result from diarrhea, as in dysentery and cholera.

**Lungs.**—The lungs exhale air which is nearly saturated with water vapor at body temperature. The rate of excretion by this channel therefore depends upon the rate of breathing, and upon the temperature and moisture of the air breathed. Benedict and Carpenter<sup>10</sup> concluded that under basal conditions the loss by way of the lungs averaged 36 per cent of extra-renal losses, or 250-350 cc./day, for adults. This water also aids in the dissipation of heat.

**Skin.**—The water losses from the skin are of two types, sensible perspiration, or sweat, and insensible perspiration. The latter goes on whether sweating is present or not.

*Sweat* is secreted when it is necessary to cool the body. Increase in body temperature from whatever cause, ordinarily accompanied by vasodilation, produces sweat. But vasodilation may occur as a result of emotional states, and sweat may result when neither body nor external temperatures are high. Humidity, air currents, clothing and exercise

affect the amount of excretion. Sweat breaks out when the external temperature rises above 30-37 °C. The surface of the body then becomes cooler. Flack and Hill<sup>29</sup> state that as much as 10 liters of water may be evaporated during a ride in the sun of the South California Desert. Increase in humidity causes sweating at a lower temperature. Rubner<sup>74</sup> found that a person would sweat at 25-26 °C. when the relative humidity was 60 per cent, and at 30° when the humidity was 22 per cent. According to Zuntz<sup>89</sup> a soldier using 1000 Cal. in a march, at 10° C., in saturated calm air, lost 800 gm. of water. Each 1 °C. increase in temperature caused an extra water loss of 38 gm.; each unit increase in wind (12 different values) lessened it 70 gm. The faster-moving air evaporates the sweat more rapidly and hence has a greater cooling effect.<sup>15</sup>

Clothing both increases insulation and prevents heat loss. The color and material of the clothing are important, and different weaves permit more or less air circulation. White silk is the favorite clothing in warm countries, and dark wool in cold climates.

Immersion in hot baths or hot-air baths have been used, largely therapeutically, to increase sweating. It has been found that a hot bath (42.4 °C.) increased sweat eighty times.<sup>73</sup>

When the heat is intense and the sweating is insufficient to cool the body, the temperature may rise to 109-110 °F.<sup>27</sup> This condition, called "heat stroke" differs from that called "heat cramps." Obviously the excretion of sweat is related not only to heat exchange, but also, because of the Na<sup>+</sup> and Cl<sup>-</sup> contained in it, to blood volume, interstitial fluid volume and renal excretion. These aspects of the physiology of sweat are discussed in Chapter 3, under *Sweat*.

*Insensible perspiration.*—It has long been known that although it is not visible, water is evaporated from the surface of the skin. Numerous tests have shown that this loss of moisture is by a process of vaporization without loss of salts. Water lost as insensible perspiration amounts normally to about 500 cc. per day. Patients have been studied who have no sweat glands, but who lose as much as 800 cc. of water from the skin daily.

*Insensible weight loss.*—Generally it has been inconvenient to study separately the water lost from insensible perspiration and that from the lungs. The usual methods are to measure water output in a calorimeter or to calculate the water from the insensible weight loss. The measurement of the water in the calorimeter by Soderstrom and DuBois<sup>81</sup> has indicated, under a variety of conditions, that an average of 24 per cent of the heat produced was expended in the vaporization of water. Therefore this datum has been used as a measure of energy metabolism.

The insensible water loss is calculated from the total weight loss by adding the CO<sub>2</sub> output and subtracting the oxygen absorbed. Johnston and Newburgh<sup>47</sup> have calculated that the water loss constitutes 82 per

cent of the loss of weight when the respiratory quotient is 1.0, 104 per cent when it is 0.707, and 92.8 per cent when it is 0.82.

As the relative humidity increases, the amount of vapor lost diminishes, and at higher temperatures this diminution is more pronounced. Wolpert<sup>58</sup> found that the hourly production of water calculated for a 70-kg. man varied from 31 to 71 gm. At 18 °C., relative humidity 35-60 per cent, seven subjects vaporized 56-60 gm./hour, or 1400 cc./day. Benedict and Carpenter<sup>10</sup> have made a very extensive study totalling over 2150 hours; they found that the insensible perspiration of men is approximately 40 gm./hour, or 960 cc./day; at 30 per cent humidity, 60 gm., at 50 per cent humidity, 26 gm./hour. Soderstrom and DuBois<sup>81</sup> found that healthy men excreted about 700 cc./day.

Rubner<sup>75</sup> determined that these losses are proportional to body weight, and not to body surface variations. Increase in moisture of the air decreases the heat lost by evaporation, but increases that lost by radiation and conduction. Increase in the temperature of the air, however, increases heat lost by evaporation and diminishes that lost through radiation.

Benedict and Root<sup>11</sup> have extended these observations and have shown that the weight loss by insensible perspiration is proportional to the energy metabolism in adult patients with diabetes and thyroid disease, as well as in normals. Levine and Marples<sup>53</sup> have made similar studies of infants and children. Newburgh and Johnston<sup>47, 65, 66</sup> have improved the method to study heat elimination over long periods, including variations of diet and exercise. These authors and Lavietes<sup>52</sup> have given formulas for calculation of the energy metabolism by estimating the water balance.

When the body temperature is rising or falling, the heat of vaporization is variable, and failure to vaporize allows increase in body temperature. The proportion of insensible weight loss is not materially altered in relation to heat production by moderate exercise or food.

Alterations in the volume of fluid in the body do not always affect the insensible loss unless there is also a change in the osmotic pressure in the body fluids; hence edema causes no increase and dehydration causes a diminution. Gilman and Barbour<sup>35</sup> showed that large amounts of water caused increased insensible loss, hypertonic saline caused diminution, and isotonic saline caused no change.

#### WATER REQUIREMENT

Practically, the water intake is a problem of minor significance, for water is available everywhere in abundance or the place is shunned by living creatures (and plants). If the individual is thirsty, he drinks. If he drinks more than is necessary the surplus will be excreted by the

kidneys. This obviously does not apply to special cases, such as water supply for ship consumption, or for desert expedition. In Bermuda, where water is limited to rainfall, or in localities where saline springs furnish the only supply, the water requirement is a matter of concern. Pathological conditions present individual problems.

In American cities the water consumption is 1000 l./person/day. Of this only one-tenth of one per cent, or 1 l./day is actually drunk.

**Sources of water intake.**—The water requirements of the body are met by (1) drinking water, (2) water in food, (3) water formed in combustion of food, (4) water of combustion of body substance. Water may be furnished parenterally in special cases. The body is indifferent as to whether water is furnished from one source or another.

**Drink.**—The amount of water consumed as such is only about one-third of the total water intake, except when the demands are very great, as under conditions of excessive heat or exercise. Ordinarily not more than 1-1.5 liters are so taken, and infants do not drink more than 100 cc. per day.

**Food.**—Some foods and beverages contain so much water that they are taken mainly for their water content. Most of our foods contain a great deal of water; meat about 70 per cent, milk about 87 per cent, and some vegetables, as cucumbers and watermelons, over 95 per cent. Even dry flour contains over 10 per cent of water. However, the water content as well as other factors may be considerably altered in preparation and cooking. Extensive data on the water content of foods as purchased<sup>5</sup> and as served<sup>55</sup> are available.

Babies taking the common dilute food mixtures and herbivora eating a diet of "greens" do not require extra water, but can live upon that in the food. In fact, if offered extra water they often refuse it.

**Water formed by combustion.**—Magnus-Levy<sup>56</sup> calculated the amount of water formed in the oxidation of foodstuffs as follows:

Grams of Water Yielded per 100 gm. of Foodstuff.

Fat .....	107.1	Protein .....	41.3
Starch .....	55.5	Alcohol .....	117.4

An ordinary diet yields about 12 gm. of water per 100 Calories. When insufficient food is supplied, the oxidation of the body substance furnishes water according to the amounts of protein, fat and carbohydrate destroyed. The above values apply to the proximate principles whether they occur in foods or in the body substance.

**Factors governing requirement.**—Empiricism has been our guide in determining water requirement. The water content of freely chosen diets plus the water voluntarily consumed has been taken as a standard. The actual requirement is governed by body weight and surface area,

food intake, temperature and humidity of the environment, and exercise. In infants the water intake is large in proportion to weight, and therefore water requirements can best be computed in terms of surface area. The amount of water required is roughly parallel to the energy metabolism, approximately 1 cc./Cal.

Table 23.—Range of Average Water Requirement of Children at Different Ages under Ordinary Conditions.\*

Age	Average Body Weight (kg.)	Total Water in 24 Hours (cc.)	Water per kg. of Body Weight in 24 Hours (cc.)
3 days	3.0	240–300	80–100
10 days	3.2	400–480	125–150
3 months	5.4	750–864	140–160
6 months	7.3	950–1130	130–155
9 months	8.6	1075–1240	125–145
1 year	9.5	1140–1300	120–135
2 years	11.8	1350–1475	115–125
4 years	16.2	1600–1800	100–110
6 years	20.0	1800–2000	90–100
10 years	28.7	2000–2440	70–85
14 years	45.0	2250–2700	50–60
18 years	54.0	2160–2700	40–50

\* White House Conference on Child Health and Protection,<sup>85</sup> page 316, Table 5. Reproduced by permission of D. Appleton-Century Co.

Standards of intake and methods for calculation of the water requirement have been given by the White House Conference Report<sup>86</sup> and Adolph<sup>2</sup> and are reproduced in Tables 23 and 24. Table 23 shows that the old pediatric rule, “one-sixth of the body weight in fluids” is not a bad one for infants. For adults the water requirement amounts to about 1/20 of the body weight.

Table 24.—Coefficients for Calculating the Water Requirement of an Individual, in cc. per 24 hours, from the Surface Area of the Body (A) and the Energy Requirement (E).\*

Path of Loss	Minimum	Average to Liberal
Growth (or storage)	0	15 A to 30 A
Urinary	400 A	1000 A to 1500 A
Fecal	30 A	90 A to 150 A
Basal extrarenal	250 A	390 A or $1.73 \times 0.25 E$ basal
Exercise (sweat)	$1.73 \times 0.4 E$ excess	$1.73 \times 0.55 E$ excess
Temperature control (sweat)	0	1800 A (atmospheric °C. – 32° C.)
Total (approximate)	2100	3400 to 5000

\* Adolph,<sup>2</sup> Table 2. Reproduced by permission of *Physiological Reviews*.

A = Surface area of the body measured in square meters.

E = Energy requirement expressed in large calories.



It can be seen from inspection of Table 24 that, except for growth, the total requirement is divided into various fractions dependent upon the excretion from different paths. The two main variables above the basal requirement are the amount of exercise and the external temperature. This, then, gives a convenient summary of the more elaborate statements of the effects of temperature, humidity, wind velocity and exercise given above. The requirements can be still more briefly computed from the common water outputs:

Urine	1000-1500 cc.
Feces	50- 200
Skin	450-1050
Sweat	100- 500
Lungs	250- 350
<hr/>	
	1850-3600 cc.

Benedict found total consumption of water ranging from an average of 2290 cc. for persons in repose to 3700 cc. for those at moderate work. Klinker<sup>49</sup> gives corresponding figures of 900-2500 and 2500-4500 cc. He feels that constitutional factors are important in determining the intake.

A generous meal (1000 Cal.), with 400 cc. of water as beverage, 600 cc. as water content of the food, and 125 cc. as the water of combustion, furnishes a total of 1125 cc.

These conditions apply to normal individuals on diets of average composition. With diets high in protein or salt these values for both intake and output are increased. Pathological conditions characterized by vomiting, diarrhea, profuse sweat, or diseases such as diabetes mellitus or diabetes insipidus do not come under this category. Fever increases the rate of metabolism approximately 13 per cent for each degree of fever, and this increases the water requirement.

**Water balance.**—Determination of water balance is as important physiologically and pathologically as is that of nitrogen or of any of the minerals. This field is of only recent development because of the difficulty of the measurements and their interpretation. Water balance may be determined in two ways, either by algebraic summation of water intakes and water losses, or by weight gains or losses. As in the case of other constituents, the importance of water loss or gain cannot be judged from the amount. A few cc. of extra water in glaucoma may destroy vision, but a 30-pound loss may result from nothing more serious than a dressmaker's dictum.

Water balance is associated not only with metabolism of the minerals, but also with that of protein, fat and carbohydrate. In growth each pound of flesh requires its quota of water as well as of protein and min-

erals. So too, when tissue is being destroyed, as in wasting conditions or starvation, not only do negative balances of nitrogen occur, but also depletion of both intracellular and extra-cellular water and their contained potassium, sodium and chloride. Benedict and Carptener<sup>10</sup> showed that with isocaloric diets, when the carbohydrate was high, 88 gm. of water per day were retained. When the diet was high in fat the same individual showed a loss of 906 gm./day. If no ketosis develops, no weight loss occurs. It has long been known that infants fed mixtures very high in carbohydrate gain weight excessively, and become flabby, due to extra water retention. Each gram of glycogen or protein retained requires the deposition of about three grams of water. There is some question as to whether the retention of fat is also accompanied by retention of water, but the best opinion is that fat replaces water.

Following Gamble's study of fasting<sup>34</sup> attempts have been made to assess water losses or gains on the basis of intracellular and extracellular water. In order to do this it is necessary to measure simultaneous balances of sodium, potassium and chloride. Gains or losses of sodium and chloride in equivalent amounts have been interpreted primarily in terms of edema or dehydration. The interpretation of water balances is still further complicated by the effects of acidosis and alkalosis. Acidosis causes negative water balance and alkalosis positive balance. High-fat diets produce ketosis, acidosis and negative water balance. It is thus very difficult to evaluate positive and negative water balances.

We will now consider how the body handles the usual intakes of fluid, and metabolizes them. Later we shall discuss the conditions in which widely varying amounts are utilized.

### CONTROL MECHANISMS

**Thirst.**—Water is consumed in response to thirst. Except perhaps in diabetes insipidus, the need of water is reflected in the drying of the mouth and pharynx. Cannon<sup>18</sup> has developed the thesis that this causes the sensation of thirst. Rabbits which had been thirsted and then given pilocarpine, which causes a flow of saliva, did not drink when water was offered, but the controls (given no drug) greedily drank 50-100 cc.<sup>67</sup> When the mouth is excessively dry there is considerable secretion of mucus. Drinking diluted vinegar and cereal water removes this secretion more effectively than plain water. Carlson<sup>19</sup> claims that other mechanisms besides dryness are operative. Thus, after eating salty food, there is a demand for water even though the mouth is moist.

**Absorption.**—Ingested water passes rapidly from the stomach. Gastric absorption is negligible. Water causes little gastric secretion. Hawk<sup>40</sup> has made numerous studies upon water drinking, especially drinking with and between meals. He found that either procedure is

without deleterious effect. The more dilute the contents of the stomach, the more rapidly does the material pass into the intestine. The temperature of the water drunk is of minor importance. Whether ice water or hot drinks are taken they reach body temperature within 20 minutes, and the subsequent course is identical.

Water taken by mouth is rapidly absorbed in the intestine and excreted by the kidneys. Water has been called the best diuretic. In healthy persons the ingestion of water causes excretion of urine within 2 hours. In infants the period of excretion is extended, but it must not be forgotten that infants consume relatively large amounts at a feeding. Water may cause a blood dilution of 10-15 per cent in 20-40 minutes. According to Klinker<sup>49</sup> this effect disappears shortly and is followed by a secondary dilution. In 4-5 hours the blood volume has again become normal. The amount of dilution is not proportional to the water intake. It may even be brought about by suggestion. The dilution must depend upon the degree of hydration of the tissues and its regulation, in part, by central stimuli. Dresel and Leitner<sup>23</sup> have shown that extra blood cells are brought into circulation. The resulting increase in blood volume can be measured by a dye method. This effect is more marked in children.

Water is not all absorbed directly by the blood stream, but also by the villi of the intestine, and passes into the lymph stream. The portion absorbed by the portal system is brought to the liver, in which intermediary storage of water occurs.<sup>50</sup> The capillaries in the liver may be quite permeable and remove enough water to regulate the blood pressure. The liver not only increases in size, but water exudes from the surface and drops into the peritoneal cavity.<sup>6</sup> In dogs in which the liver is isolated, Kunz and Molitor<sup>51</sup> saw the water dropping from the surface of the liver every time water was ingested. Mautner<sup>59</sup> says this fluid contained the same concentration of chloride and calcium as the blood, but was higher in sugar. Hence in a true sense the liver may be said to be the emergency regulator of the blood and water volume.<sup>22</sup> There is also a nervous control of this mechanism. The effect of the liver may be demonstrated not only as has been mentioned above, but also when the liver is put out of commission by an Eck fistula or by blocking of the reticulo-endothelial system. Such a liver shows a much lessened ability to hold back ingested water.

Water is deposited primarily in the skin and subcutaneous tissues and muscles.<sup>24, 50</sup> After water was given to thirsting animals, as much as 78 per cent was accounted for by increase of the water content of the skin.<sup>78</sup> In infants the mechanism is more extensible and the storage of longer duration. A condition called "hydrolability" exists, in which as much as a kg. of water may be stored for several days, primarily in the skin, and then gradually excreted.

*Extra-oral fluid.*—When vomiting or intestinal obstruction contraindicates taking fluids by mouth, enemata are often given. Their main function is to furnish water and minerals. Surprisingly large amounts of fluid can be introduced by the rectum, enough to meet the body's need for fluid, especially if small amounts are given at a time.

Solutions given into fistulae or isolated intestinal loops are rapidly absorbed, whether hyper- or hypotonic.

Fluid may be injected subcutaneously, intraperitoneally, intramuscularly, or intravenously. Water is never used for this purpose, but usually saline or some modification of Ringer's solution. Water given subcutaneously does not act as a diuretic, for it is slowly absorbed. If either hypotonic or hypertonic saline solutions are given, they are brought to the osmotic concentration of the body fluids before absorption.

Smith and Mendel<sup>79</sup> injected sufficient isotonic solution of various neutral salts to double the blood volume. In every case most of the fluid passed into the tissues within five minutes without causing edema. From the tissues it passes back into the blood stream to be excreted.<sup>17</sup> Even in experimental uranum or chromate nephritis Chisolm<sup>21</sup> found that injected isotonic fluids rapidly left the blood stream.

Hypertonic solutions given intravenously act as powerful diuretics.

**Nervous and hormonal control.**—The central and hormonal control of body fluids affects the volume of urine and sweat. (See Chapter 3, under *Sweat*, and Chapter 4, *Internal Secretions*.) Of greatest importance is the antidiuretic effect of pituitrin. But there are also more general relations. The thyroid and parathyroid glands, the spleen and pancreas have some control of the body water. Activity of the first three is supposed to lower body water, and insulin, perhaps mainly through its dissipation of ketosis, to increase body water. The adrenal gland also has a fundamental action. Adrenalin acts first as a vasoconstrictor and then as a vasodilator. The cortical hormone profoundly affects the sodium, chloride and potassium metabolism, and hence the body water.

Castration produces not only differences in the body form and increase in adipose tissue, but also an increase in body water. Testicular extract is supposed to act as a diuretic for eunuchs.

**Allergy.**—Sensitivity to proteins or other specific chemical substances may result in either water loss or water retention. In the first class comes hay fever, with increased nasal and lacrimal secretion and diarrhea. The second category includes angioneurotic edema and urticaria. Perhaps tuberculous pleurisy should be so classed.

**Diuretics.**—Little need be said here of the action of diuretics on increasing the excretion of water by the kidney. Treatises on pharma-

cology should be consulted. We have already indicated the importance of the three main diuretics, water, salts and nitrogenous end products. Further, the effects of hormones have been mentioned.

Only three of many diuretic drugs need be included. The purines undoubtedly have a strong diuretic effect. This is due in part to action on the heart and blood vessels. It has been shown that they mobilize sodium and chloride in the blood and cause blood dilution by drawing water from the tissues. Hence their effect is extrarenal. Presumably mercury salts also affect the mobilization of body water.

The action of purines is less enhanced by acids than is that of mercury salts. Blumgart *et al.*<sup>14</sup> have shown that 90 per cent of such water is from interstitial body water. Digitalis has long been known to be effective in causing diuresis in the edema of heart disease. Its principal diuretic effect depends upon improvement in the circulation.

#### RELATION OF MINERAL INTAKE TO WATER METABOLISM

The relations of minerals to water metabolism may be divided sharply into two categories: the first, in which water is taken with food, and the second, in which minerals are taken in the water.

**Minerals in food.**—*High mineral intake.*—The relation of high and low mineral intakes to metabolism involves first the body's primary needs for maintenance and growth, which are discussed in Chapters 2 and 14. Secondly, the amount of substance to be excreted in the urine is determined especially by the amounts of sodium, potassium, chlorine and protein ingested. The higher the intake the greater is the volume of urine formed.

Gamble *et al.*<sup>33</sup> have shown that rats will consume daily about 10 per cent of their weight in water upon ordinary dry laboratory diet, but when about 17 per cent of sodium chloride is added to the same diet, they drink over half their body weight in water every day. (See also Chapter 3.) Water drinking of the same order can be observed in lactating rats and mice. This is because of the increased protein and mineral intakes which supply the increased metabolic needs and the materials for the formation of milk. The above conditions apply when water is furnished *ad libitum*. But the mineral intakes must always be considered in relation to water available or consumed. If little water is available, high mineral intakes are dehydrating.

Addition of sodium chloride to the diet of a normal individual without change of liberal water intake may induce salt and water retention. As little as 10-15 mg./day may have this effect. With nephritics the retention is greater.<sup>13</sup> Meyer<sup>61</sup> has shown that children are especially susceptible. They act like nephritics and become edematous. Rominer<sup>72</sup> has shown that they may store salt without water.

Various salts act differently. The anions follow the Hofmeister series as diuretics. Sodium is associated with water retention. Calcium and potassium are diuretics. Acids remove water from the body and alkalosis is associated with edema. Alkaline salts *in vitro* cause a transfer of  $\text{Cl}^-$  and water from blood cells to plasma with a shrinking of cells. Peters had difficulty in demonstrating such an effect in a normal adult after 40 gm. of sodium bicarbonate by mouth.

*Low mineral intake.*—Animals and man can adapt themselves to diets which are very low in minerals. Certain African tribes live in regions where sodium chloride is almost absent. It is, however, very highly prized, and they make extreme efforts to obtain it. Animals cannot grow on mineral-poor diets (see p. 319). When individuals are placed abruptly on a low sodium chloride intake a rapid loss of body water is encountered and the urine becomes almost free of  $\text{Na}^+$  and  $\text{Cl}^-$ . Provided that the protein intake is adequate these persons still need the same amount of water for excretion in the urine. Thus, water ingestion may cause either water excretion or retention, or mineral excretion, depending upon the mineral and water content of the previous diet.<sup>45</sup>

*Minerals in water.*—When minerals are consumed in the water the body must make internal adjustments between the mineral and water metabolism. Minerals consumed in the water may be deleterious. People shipwrecked at sea find "water, water everywhere, nor any drop to drink." The salinity of the ocean is about 3 per cent. The consumption of such water leads to death. After drinking 1500 cc. of water containing 1.65 per cent sodium chloride, only 200-300 cc. of urine were excreted in 4 hours. Even in 12 hours equilibrium had not been reestablished.<sup>49</sup>

The waters of certain wells in the southwestern part of the United States are not potable. The rivers and wells from this "alkali region" contain  $\text{Na}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{--}$  in amounts sufficient to kill vegetation along their shores. Analyses have shown the content to be 0.5-5.0 per cent, and in certain instances in excess of 20 per cent. Human beings find that these waters taste bitter and act as bladder and intestinal irritants. Stockmen attribute poor condition or death of cattle to this source. Heller and Larwood<sup>41, 42</sup> have studied the effect of such saline solutions upon rats fed an adequate diet and no other water. They used 0.5-5.0 per cent solutions of  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{MgSO}_4$ ,  $\text{NaHCO}_3$  and saturated  $\text{CaSO}_4$ , and also mixtures of these salts. They found that 1.5 per cent  $\text{MgSO}_4$  had a retarding effect upon growth, and increased the mortality rate of the young; 2.5 per cent had a decided toxic effect.  $\text{CaCl}_2$  was even more harmful: no normal young were found when the intake was 1 per cent; 1.5 per cent lowered the growth rate; and 2.5 per cent caused death. One per cent  $\text{NaCl}$  diminished reproduction; 2 per cent retarded growth, and 2.5 per cent induced weight loss.  $\text{NaHCO}_3$

was less harmful than  $\text{Na}_2\text{CO}_3$ . Two per cent  $\text{NaCl}$  plus 0.5 per cent  $\text{CaCl}_2$  or  $\text{MgSO}_4$  inhibited growth.

Salts in combination seemed as deleterious as singly. Animals could tolerate gradual increases better than sudden shifts to salty drinking water. The fluid intake increased as the salinity became greater "up to the point where the animal preferred to die of thirst rather than drink the necessary amount."

Allison<sup>3</sup> reported that the waters of one-fourth of the state of Minnesota may show 0.1-1.0 per cent of minerals, chiefly  $\text{SO}_4^{--}$ . Similar conditions are found in Montana and the Dakotas. The condition is a serious one for cattle. They show a run-down, ragged appearance and many weaken and die prematurely. The bones become soft and the animals show a perverted appetite.

*Mineral waters.*—The therapeutic and physiological effect of mineral waters is a problem complicated by many factors. A "cure" usually takes place at a spa, which has enjoyed a reputation, perhaps worldwide, as a place where health may be attained. Some of these resorts have been famous since the dawn of history, certainly before the Roman days, and are coupled with miraculous cures in folk-lore. In modern days these resorts comprise large medical institutes well equipped not only for general therapy but for special physical therapy as well as hydrotherapy, and conducted by corps of competent medical practitioners.

A patient goes to such a place confident that he will attain great benefit. He is located in a pleasant environment which supplies change from his usual activities and worries, and entails also physical rest, mild exercise and recreation. Under such conditions it is not surprising that drinking the waters of the mineral spring produces benefit. Perhaps the consumption of water itself is important, and drinking 7 or 8 glasses of warm "aqua distillata" under similar conditions would result in physiological well-being. Added to this, regular life, regular habits, cathartics and a carefully controlled diet would constitute an armamentum not to be held lightly.

No small part of the efficacy of mineral waters may, perhaps, be ascribed to psychotherapeutic effects. Part of the claims of their value come from those financially interested, and part are due to the gullibility of a credulous public eager to grasp at any straw to regain lost health. There is no nostrum or quackery without its testimonials, many of which may be honest. But you "cannot fool all of the people all of the time." The fact remains that there is too much evidence from too many sources, scientific and otherwise, to be dismissed on such a basis. The specific values of different waters are undetermined. They may lie in the presence of radioactive materials, or of the rarer elements in traces, which we have recently learned to respect, or in some unusual salt complex

such as "active" iron. The value of spas must at present be accepted as intelligent empiricism, above and beyond all the factors for well-being which we have enumerated.

## WATER INSUFFICIENCY AND EXCESS

### Insufficiency

**Low intake.**—Certain animals, notably flour moths, live under conditions in which the water content of the diet is not more than 10 per cent. No mammalian species approach such water economy.

A certain amount of water is required for the deglutition of dry food. Part is provided by the saliva and part by water ingestion. Bing and Mendel<sup>12</sup> showed that with ordinary dry diets mice drank 1.3 cc. of water for every gram of food eaten. If the water was restricted beyond this point less food was consumed. If no water was supplied they ceased eating and died in a few days. Hence it is impossible to study water deprivation without food limitation.<sup>46</sup> If no water is given, animals are restricted to the metabolic water produced by the breakdown of tissue. Voit<sup>84</sup> pointed out that thirst was less in complete starvation than when dry food was eaten without water.

Lack of water causes death before lack of food. Rowntree<sup>73</sup> cited a case of an Italian political prisoner who died as a result of abstinence from food and water for eighteen days. He suffered little from hunger after the first day, but experienced terrible thirst until the end. In the desert death occurs in 36-72 hours. Animals have borne complete starvation for 28 days and recovered. One of Poletaëw's<sup>71</sup> dogs survived 22 days without water, with a 47 per cent loss of weight, and in a subsequent starvation died with a 60 per cent weight loss. Further data on lack of water are found in Morgulis' monograph.<sup>64</sup>

**Dehydration.**—Diminution of body water is associated with a hemoconcentration.<sup>48</sup> Normal conditions are rapidly restored when water is taken; no diuresis ensues until the deficits are made up. Marriott<sup>58</sup> used the term "anhydremia," for he claimed that no serious desiccation occurs unless the water content of the blood is diminished. But Gamble and McIver<sup>32</sup> were able to show that significant concentration of blood took place only when the extracellular fluid had been almost completely exhausted. Dehydration occurs when the water outgo is greater than the intake. It may be produced by low intake, diuresis, sweating, vomiting, fistulae or diarrhea. The nature of the body water loss, its distribution between intracellular and extracellular water, and the concomitant changes in minerals and osmotic pressure in dehydration have been described in Chapter 3. Metabolism studies have shown that when dehydration results from limited water intake, first the interstitial water is reduced and then the cell water.<sup>87</sup> Adolph<sup>1</sup> produced dehydration by



ingestion of large amounts of urea or sodium chloride with limited water intake.

Inanition or salt fever is a term used to designate the dehydration which occurs in new-born children. This was first described by Holt, in 1895, who showed that water caused the symptoms to disappear. Finklestein<sup>28</sup> showed that fever could be induced by salt and sugar.

Diuresis which leads to intense dehydration has been produced by intravenous administration of hypertonic solutions, in man and animals.<sup>7</sup> Fever up to 125.6 °F. has been attained with salt and lactose.<sup>4, 8</sup>

Ingested acids or acidifying salts cause not only acidosis but also dehydration (see pp. 201 and 287).

Dehydration occurs in infants after severe vomiting and diarrhea, accompanied by acidosis. In Germany the condition is called "alimentary intoxication."<sup>28, 83</sup>

High-fat diets which produced ketosis were found to be of benefit in the treatment of epilepsy. Ketogenic diets cause not only acidosis but also dehydration.<sup>68</sup> Subsequently dehydration produced by limitation of water intake has come to be used in the treatment of this disease.<sup>26</sup> Manchester, Husted and McQuarrie<sup>57</sup> found, in a study of four epileptic children, that dehydration reduced the insensible perspiration below that expected, although the energy metabolism was normal. Conversely superhydration, as has been found by others, increased the insensible perspiration.

### Excess

**Water Intoxication.**—When more water is given than an individual can excrete, sweating ensues, accompanied by headache, nausea, weakness and incoördination.<sup>62</sup> Weir, Larson and Rowntree<sup>85</sup> produced a similar condition in dogs and called it "water intoxication." They stated that the ingestion of large quantities of water, 50 cc./kg., with or without pituitary extract, resulted regularly in the development of the following train of symptoms: asthenia, restlessness, frequency of urination, diarrhea, nausea, retching, vomiting, tremor, salivation, muscle twitching, convulsions tonic and clonic, frothing at the mouth, stupor and coma. Death ensued when the water administration was continued after the onset of convulsions, whereas complete recovery resulted within twelve hours in a large proportion of the animals if no more water was given. This was not accompanied by a change of temperature, by edema, or by marked increase in plasma volume.<sup>89</sup> Blood pressure and intracranial pressure were increased. The vomitus contained large amounts of Cl<sup>-</sup>, and a dilution of blood Cl<sup>-</sup> and lowering of osmotic pressure resulted.<sup>80</sup> Intravenous hypertonic salt solutions relieved the symptoms.

**Edema.**—The pathology of increased water retention lies beyond the scope of this treatise. Water may be retained in the tissues or in any of the body cavities, the peritoneum, pleural cavities, joints or bursae. In edema so much water accumulates in the tissues that they pit on pressure.

The main causes of edema are interference with the circulation, as in heart disease; local obstruction to the return of blood or lymph, as in liver disease or elephantiasis of the limbs; low oncotic pressure, as in kidney disease; or hypothyroidism. Most of these are related either to capillary pressure or to diminished oncotic or osmotic pressure in the capillaries. Local water retention may be caused by chemical or mechanical irritants such as mosquito bites and pressure blisters.

A condition called low-protein edema may result from faulty diet. This occurred in Central Europe during the World War and was called war edema. It was subsequently shown that diets low in protein and high in bulky vegetables produce similar conditions in man and experimental animals.<sup>31, 54</sup> The plasma protein is diminished. Similarly, in experimental removal of plasma proteins, or in nephrosis, in which low plasma proteins are found, edema regularly ensues. This may be referred to the low oncotic pressure of the blood plasma.

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## Chapter 13

### Anion-Cation Relationships

There has been a good deal of confusion in writing about the acid-base economy of the body because of the looseness of terms used. The term *acid-base equilibrium* has been reserved, in this book, to characterize the proportions of the various species of anions and their acids which determine the pH of biological fluids. The maintenance of acid-base equilibrium has been shown to be as fundamental to life as the regulation of water, temperature or specific ion concentrations. The neutrality of the body is stabilized by buffer and equilibrium mechanisms and by the processes of excretion. The problem of how the acid-base equilibrium is maintained has received extensive treatment in recent monographs <sup>4, 16, 29, 35, 45, 51, 56</sup> and is outlined only briefly here. Minor departures from the normal equilibrium are quite common, but derangements are infrequently extreme enough to cause death. No attempt will be made to discuss the clinical pathology of acidosis or alkalosis except insofar as it throws light on the normal mechanisms.

It is profitable to discuss also the related subject of the excess of only the mineral cations, anions, or ionogens which occurs in biological systems. This procedure was used to describe the mineral content of the body (Chapter 2) and of the secretions and excretions (Chapter 3). In this chapter it will be extended to cover the analysis of food. This is of importance because the minerals ingested determine the extent to which the body defenses must operate, and the accumulation or depletion of mineral reserves.

Finally it is desirable to discuss what we have called *cationogen-excess balance*, i.e., the excess or deficit of the total positive or negative mineral equivalents of the intake compared to the output. This balance is dependent, in the final analysis, upon the minerals in the food.

#### MECHANISM OF ACID-BASE EQUILIBRIUM

##### Blood

**Bicarbonate buffer system.**—*Determination of pH.*—The slight alkalinity of the blood serum is maintained with great tenacity, and in life does not change greatly from the normal of pH 7.35-7.43. The acid-

base equilibrium of blood serum is usually calculated from the well-known Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}'_1 + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} \quad (1)$$

In this equation  $\text{pK}'_1$ , the apparent first dissociation constant of carbonic acid, has a value in serum, at 38 °C., of 6.10.\*  $[\text{CO}_2]$  or  $[\text{dissolved CO}_2]$  represents anhydrous or free  $\text{CO}_2 + \text{H}_2\text{CO}_3$ . If any two of the three variables ( $\text{pH}$ ,  $[\text{HCO}_3^-]$ , or  $[\text{CO}_2]$ ) are known, the third can be calculated.

The  $\text{pH}$  of the serum may be measured either electrometrically, by the hydrogen or quinhydrone electrode or glass electrode; or colorimetrically, with indicators; or it may be calculated from equation (1). This last method requires that blood be equilibrated at a known  $\text{CO}_2$  tension and temperature, which fixes the value of the  $[\text{CO}_2]$ . Then the sum of the  $[\text{HCO}_3^-]$  and  $[\text{CO}_2]$  is measured, usually by the gasometric methods developed by Van Slyke. When the known  $[\text{CO}_2]$  is subtracted from the total, the difference represents the  $[\text{HCO}_3^-]$ , and one is enabled to calculate the  $\text{pH}$ . The convenient expression in  $\text{mM/l.}$  of both  $[\text{HCO}_3^-]$  and dissolved  $[\text{CO}_2]$  not only states them in the common terms necessary for calculation of the  $\text{pH}$ , but also makes evident their relation to the other minerals in the blood, when these are calculated in similar terms. The normal value of the  $[\text{HCO}_3^-]$  in the serum of venous blood, separated without contact with air, is about 61 vol. per cent, or 27  $\text{mM}$ , and the  $\text{CO}_2$  tension is about 40  $\text{mm.}$ , or 3 vol. per cent, or 1.35  $\text{mM}$  of dissolved  $\text{CO}_2$ . The ratio of  $[\text{HCO}_3^-]$  to  $[\text{CO}_2]$  is thus about 20/1, and the logarithm of this value is 1.3. It follows, according to the Henderson-Hasselbalch equation, that  $\text{pH} = 6.10 + 1.3 = 7.40$ .

The alveolar  $\text{CO}_2$  tension with which the blood is in equilibrium may be measured; or, if the  $\text{pH}$  and  $[\text{total CO}_2]$  are known, the  $[\text{HCO}_3^-]$  and the  $[\text{CO}_2]$  (which is a function of  $\text{CO}_2$  tension, temperature and ionic strength) may be calculated.

It is possible to plot these three variables so that with a knowledge of two the third may be found directly without calculation. A number of different forms of plotting are available.<sup>32, 37, 55, 73, 76</sup> Peters' form

\* The studies by Stadie and O'Brien<sup>66</sup> on the hydration of  $\text{CO}_2$  to  $\text{H}_2\text{CO}_3$  enable one to measure the true dissociation constant of  $\text{H}_2\text{CO}_3$ :

$$\text{pH} = \text{pK}'_{1(\text{H}_2\text{CO}_3)} + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$$

The value of  $\text{pK}'_{1(\text{H}_2\text{CO}_3)} = 3.7$ , which makes carbonic acid a relatively strong acid. Only about 1/700 of the dissolved  $\text{CO}_2$  is hydrated to form  $\text{H}_2\text{CO}_3$ . For physiological work it is quite as accurate and more convenient to use the apparent dissociation constant and  $[\text{dissolved CO}_2]$ . The recent review by Roughton<sup>67</sup> has summarized this material.

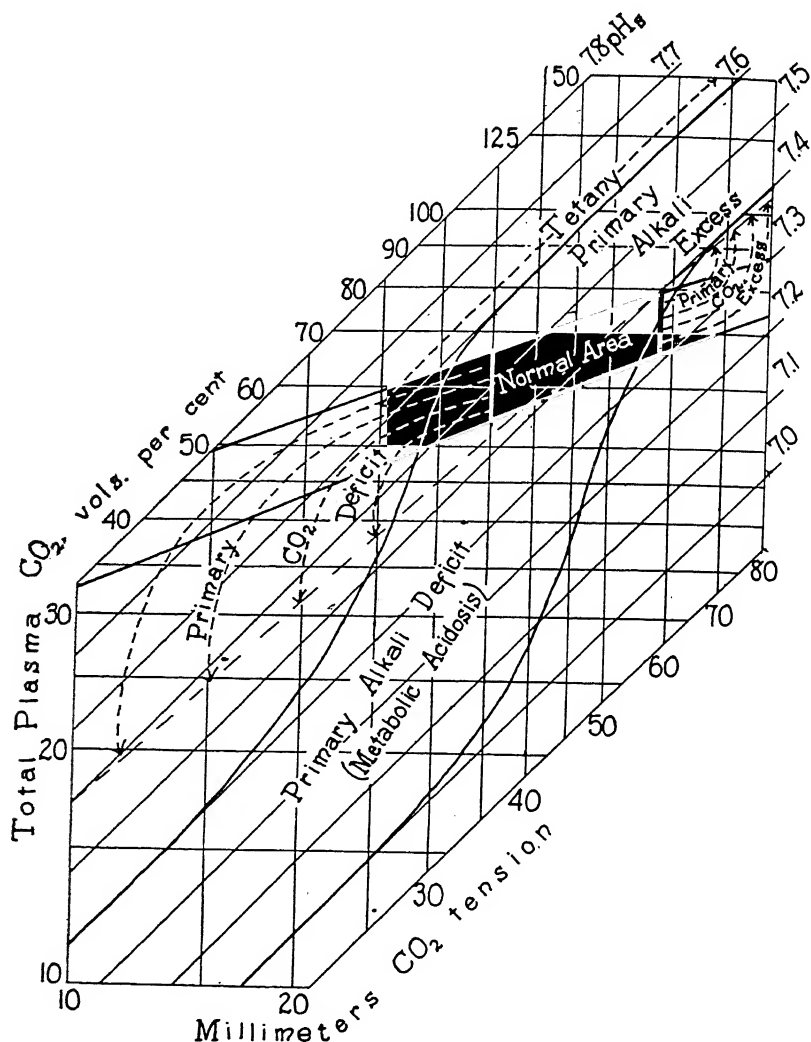


FIGURE 12. Acid-Base Equilibrium of the Blood Plasma.\*

\*Peters and Van Slyke<sup>55</sup> p. 944. Reproduced through the courtesy of Williams and Wilkins Co. Changes in CO<sub>2</sub> content, pH and CO<sub>2</sub> tension of the blood plasma in conditions of primary CO<sub>2</sub> excess and deficit.

of plotting is reproduced in Figure 12. This shows the relationship between  $[\text{HCO}_3^-]$ , CO<sub>2</sub> tension and pH which characterize the acid-base equilibrium of the blood. In order to obtain a complete picture of the electrolytes in the blood, it is necessary to plot, in addition, the

other six variables, per unit of volume— $\text{H}_2\text{O}$ ,  $\text{Cl}^-$ ,  $\text{O}_2$ , Hb, hemoglobinate and proteinate, as has been done by Henderson.<sup>35</sup>

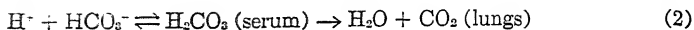
*Absorption curves.*—The  $\text{CO}_2$  tension and state of oxygenation of the blood obtained by venupuncture may vary considerably. In order to obtain a more precise value of the acid-base equilibrium of the serum, the alkali reserve or the absorption curve, in which these conditions are fixed, may be determined. For the former, the blood is equilibrated in a tonometer with mixtures of air or nitrogen and  $\text{CO}_2$  at 40 mm. tension, and for the latter at various and definite  $\text{CO}_2$  tensions such as 30, 40, and 60 mm., at  $38^\circ\text{C}$ . It is usually best to make such studies upon true plasma, that is, plasma removed without contact with air from oxygenated or reduced whole blood which has been saturated at definite  $\text{CO}_2$  tensions. The values of the normal absorption curve are given in Fig. 12, and also the normal variations in  $[\text{HCO}_3^-]$ ,  $\text{CO}_2$  tension and pH. The slope of the curve is determined primarily by the  $[\text{Hb}]$  of the blood. In general the height of the curve determines the cation content of the serum and has been used as a measure of acidosis. A comprehensive discussion is given by Peters and Van Slyke.<sup>56b</sup>

The  $[\text{HCO}_3^-]$  in blood plasma at a given  $\text{CO}_2$  tension results from the interaction between cells and plasma, and hence serum removed from blood and subsequently exposed to increasing  $\text{CO}_2$  tensions does not increase in  $[\text{HCO}_3^-]$ . The  $[\text{HCO}_3^-]$  in the plasma depends upon the  $\text{CO}_2$  tension of the blood at the time when the plasma was separated from the blood. In whole blood the increase in  $[\text{HCO}_3^-]$  with increasing  $\text{CO}_2$  tension is proportional to the amount of hemoglobin present. Therefore absorption curves in the hands of an expert furnish very precise information as to the acid-base equilibrium of the blood, but require considerable skill in manipulation and considerable knowledge for correct interpretation. Taken in conjunction with clinical information the single determination of the total  $\text{CO}_2$  of the venous plasma obtained without stasis and without contact with air is adequate for most clinical purposes.

*Addition of acid.*—Because of the buffer action of the blood the addition of acid does not change the pH markedly (see p. 283). A buffer system has been defined as a weak acid partially neutralized with a strong alkali (it has been customary to refer to this as a mixture of a weak acid and its salt). At any given concentration of total acid (*i. e.*, acid plus its anion) buffers stabilize the acidity best when the weak acid and its anion are present in equal amounts. The  $\text{HCO}_3^-$  in the blood provides still further resistance to change in pH when acid is added. The bicarbonate-carbonic acid system is an efficient one because, although the  $[\text{HCO}_3^-]$  and  $[\text{CO}_2]$  are disproportionate, the  $\text{CO}_2$  formed when acid is added escapes from solution and is removed by the lungs. This



is evident from an example of the addition of acid to the blood plasma as follows:



The normal values of the bicarbonate system in the blood plasma are, as calculated from equation (1):

$$\begin{aligned} [\text{HCO}_3^-] &= 27 \text{ mM}; [\text{CO}_2] = 1.3 \text{ mM}; [\text{HCO}_3^-]/[\text{CO}_2] = 20; \\ \log \text{ of } 20 &= 1.30; \text{pH} = 6.10 + 1.30 = 7.40. \end{aligned}$$

If enough acid were added to neutralize half the bicarbonate in equation (2), and the  $\text{CO}_2$  did not escape from the lungs, the above values would change as follows:

$$\begin{aligned} [\text{HCO}_3^-] &= 13.5 \text{ mM}; [\text{CO}_2] = 1.3 + 13.5 = 14.8 \text{ mM}; \\ [\text{HCO}_3^-]/[\text{CO}_2] &= 0.91; \log \text{ of } 0.91 = \bar{1}.96 = -.04; \\ \text{pH} &= 6.10 - .04 = 6.06. \end{aligned}$$

Such a pH is incompatible with life. If, however, the  $\text{CO}_2$  formed in equation (2) escaped, and the initial tension of  $\text{CO}_2$  remained unchanged, the initial values would be altered as follows:

$$\begin{aligned} [\text{HCO}_3^-] &= 13.5 \text{ mM}; [\text{CO}_2] = 1.3 \text{ mM}; [\text{HCO}_3^-]/[\text{CO}_2] = 10; \\ \log \text{ of } 10 &= 1.00; \text{pH} = 6.10 + 1.00 = 7.10. \end{aligned}$$

But this value is still far from the normal. The pH is regulated physiologically by the respiratory center in the medulla which controls the  $\text{CO}_2$  tension. By increasing the ventilation it may diminish the  $\text{CO}_2$  tension to a point below its initial value. If the  $\text{CO}_2$  tension is decreased one-half, the ratio of  $[\text{HCO}_3^-]/[\text{CO}_2]$  would be the same as the original value, and hence the pH would remain unaltered at 7.4. Such complete compensation usually does not take place. With the  $\text{CO}_2$  tension reduced to 1.0 mM:

$$\begin{aligned} [\text{HCO}_3^-] &= 13.5 \text{ mM}; [\text{CO}_2] = 1.0; [\text{HCO}_3^-]/[\text{CO}_2] = 13.5; \\ \log \text{ of } 13.5 &= 1.13; \text{pH} = 6.10 + 1.13 = 7.23. \end{aligned}$$

This represents the condition found in severe acidosis.<sup>33, 34</sup>

The absorption of  $\text{CO}_2$  from the tissues represents the addition of acid to the serum as in equation (2). Similar reactions take place inside the red blood cells, but, owing to the lack of interchange of mineral cations between cells and serum, a shift of anions— $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$ —and water takes place with a change in acid-base equilibrium.

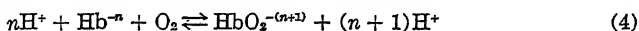
**Other buffers.**—The buffering capacity of the body fluids other than that given above depends upon the phosphate and proteins, and may be represented by the following equation:

$$\text{pH} = \text{pK}_2 + \log \frac{[\text{HPO}_4^{--}]}{[\text{H}_2\text{PO}_4^-]} = \text{pI} + \frac{[\text{Pr}^-]}{\beta[\text{Pr}]} \quad (3)$$

In the above equation the symbols represent the concentrations of secondary (alkaline) phosphate and primary (acid) phosphate, and  $pK_2$  is their dissociation constant (6.8);  $[Pr^{-n}]$  represents the meq. of proteinate; the buffer constant for the particular serum of blood (*i. e.*, the change in meq. of  $[Pr^{-n}]$  for one unit of pH);  $[Pr]$ , the concentration of protein of the blood or serum (for serum the unit is 1 gm., for hemoglobin 1 mM); and  $pI$ , an empirical constant for a single protein close to, but not necessarily identical with, the pH of the isoelectric point. This is adapted from equation 4a of Austin and Cullen<sup>4</sup> which was developed from Van Slyke's buffer equation.

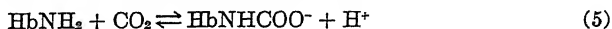
The phosphates are unimportant in the buffering of the blood plasma because they are present normally only to the extent of 1 mM/l. In the tissues the phosphates probably have a much more important role, but the nature of the compounds and their dissociation are not as yet well defined.

**Hemoglobin.**—The transport of  $CO_2$  by the blood and its expiration by the lungs are brought about chiefly by the oxygenation and reduction of hemoglobin, whose remarkable properties make this possible. Hemoglobin is a protein which acts like a weak acid on the alkaline side of its isoelectric point, and forms anions within the red blood cells. When oxygenated, hemoglobin becomes a stronger acid and dissociates into more anions at the same pH. This causes the formation of more  $H_2CO_3$ . This may be expressed as follows, for a given pH:



The  $H^+$  thus released combines with  $HCO_3^-$  to form  $H_2CO_3$  inside the red cell. When oxyhemoglobin is reduced to hemoglobin the reverse takes place. This change of strength of acid facilitates the excretion of  $CO_2$  by the lungs and the uptake of  $H_2CO_3$  in the tissues. Furthermore, the effect of  $H_2CO_3$  in the tissues is to liberate the oxygen from the oxyhemoglobin and supply it to the tissues. The  $CO_2$  can be transported without changing the pH more than 0.02-0.03.<sup>56a</sup>

In addition to the conveyance of  $CO_2$  as  $HCO_3^-$  owing to the reduction of hemoglobin, a part of the  $CO_2$  is carried bound directly to the hemoglobin. This combination, called carbhemoglobin, was suspected by Henriques,<sup>39</sup> Roughton<sup>57</sup> and Stadie<sup>68</sup> have studied the union of  $CO_2$  with amines to form carbamino-compounds or carbamates. The combination with hemoglobin may be represented as follows:



This reaction takes place rapidly with many amines. Reduced blood takes up more non-bicarbonate  $CO_2$  than oxygenated blood. Roughton calculates that, physiologically, 20 per cent of the  $CO_2$  is carried as carbhemoglobin.

There is a possibility that  $\text{CO}_2$  (and other anions) may be united to hemoglobin, even on the alkaline side of the isoelectric point. Roughton calls this  $\gamma$ -bound  $\text{CO}_2$ . The presence of such compounds of physiological importance remains unproved to date.

In its various functions hemoglobin is involved in the transport of 92-97 per cent of the  $\text{CO}_2$ .

It was shown by Henriques in 1928<sup>38</sup> that the speed of the transformation of  $\text{H}_2\text{CO}_3$  into  $\text{CO}_2$  is not rapid enough to account for the amount evolved during the passage of blood through the lungs. Roughton calculated that the reaction *in vivo* is 150 times as rapid as that *in vitro*. This difference is accounted for by the presence of an enzyme which has been found in the blood. Carbonic anhydrase, which brings about this transformation from  $\text{H}_2\text{CO}_3$  to  $\text{CO}_2 + \text{H}_2\text{O}$ , was found by Meldrum and Roughton<sup>50</sup> and by Stadie and O'Brien.<sup>69</sup> Preparations have been made which are 2000 times more active than that found in normal blood.

**Donnan equilibrium.**—When solutions of electrolytes are separated by a membrane on one side of which there is an ion which does not permeate the membrane, there is an unequal distribution of the other ions present.<sup>42</sup> The relations of the permeating ions may be expressed by the following equation:

$$[\text{A}^-]_1 [\text{B}^+]_1 = [\text{A}^-]_2 [\text{B}^+]_2 \quad (6)$$

$[\text{A}^-]$  and  $[\text{B}^+]$  represent concentrations of anions and cations respectively, and subscript figures refer to the sides of the membrane. Blood cells and plasma constitute a system analogous to this. But the blood cells contain a larger amount of proteinate than the plasma, and hence yield more anions from this source, and the cell membrane is impermeable to proteinate. Moreover the membrane is also impermeable to cations. Therefore permeating anions must be distributed unequally between cells and plasma in order that osmotic and ionic equilibrium may obtain. Due to the constraints imposed by the body it is necessary that equilibrium be reached by the transfer of anions,  $\text{H}^+$ , and water. In this discussion we have neglected the small differences in potential and in osmotic pressure on the two sides of the membrane.

A corollary of the Donnan equation is that the ratio of concentration within to the concentration without the cells is the same for the various anions. (Strictly speaking these relations should be calculated in terms of activities rather than of concentrations.) Applied to the blood, this is represented by the following equation:

$$\frac{[\text{Cl}^-]_{\text{cells}}}{[\text{Cl}^-]_{\text{serum}}} = \frac{[\text{HCO}_3^-]_{\text{cells}}}{[\text{HCO}_3^-]_{\text{serum}}} \quad (7)$$

When oxyhemoglobin is reduced the  $[\text{HCO}_3^-]$  within the cells is increased. If then, the distribution of  $\text{HCO}_3^-$  is altered, the  $\text{Cl}^-$  dis-

tribution must shift in a corresponding manner. According to the Donnan equation the ratio of the  $[\text{HCO}_3^-]$  within the cells to that of the serum should be the same as that of the  $[\text{Cl}^-]$  within and without the cells, if both are completely ionized. In consequence, the osmotic pressure within the cell rises. To compensate for this, water must pass

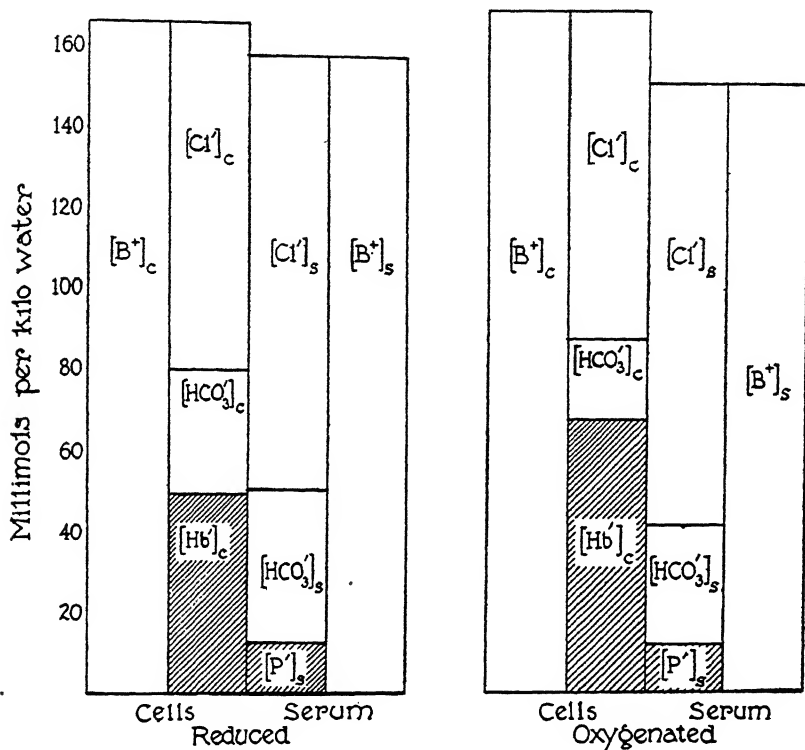


FIGURE 13. Effect of Oxygenation on the Acid-Base Equilibrium of the Blood.\*

\* Van Slyke,<sup>74</sup> p. 51. Reproduced through the courtesy of J. B. Lippincott Co.

These charts show that the effect of oxygenation (at a constant serum pH of 7.4) is to increase the union value of hemoglobin, to reduce  $[\text{HCO}_3^-]$ , to shift  $\text{Cl}^-$  and water from cells to serum.

from the serum to the cells and causes swelling of the latter. If there were a free passage of cations in and out of the red blood cells the Donnan theory would require the osmotic pressure to be greater where the protein concentration was greater. However it has been found experimentally that, at the same pH, in reduced blood the ratio of the  $[\text{HCO}_3^-]$  within to that without the cells is greater than of the  $[\text{Cl}^-]$ . In oxygenated blood the difference is less marked. This is due in large

part to that fraction of  $\text{CO}_2$  bound to hemoglobin to form carbhemoglobin, and its alteration with oxygenation. It has been stated above that 20 per cent of the  $\text{CO}_2$  is not  $\text{HCO}_3^-$ , but is in the form of carbhemoglobin. Therefore the  $\text{CO}_2$  found by analysis within the cells is present in two different forms. When this fact is properly taken into account the ratios correspond much more closely to the distribution predicted by the theory in equation (7).<sup>18</sup>

The simultaneous changes in the  $[\text{Cl}^-]$ ,  $[\text{HCO}_3^-]$  and  $[\text{H}_2\text{O}]$  are best expressed by the diagram reproduced in Figure 13. The experimental data on the distribution ratios are given in Table 25. This shows the ratios for  $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$  of that in the cells to that in

Table 25.—Ratios of Distribution of  $[\text{Cl}^-]$  and  $[\text{HCO}_3^-]$  Between Cells and Serum.\*

	Reduced Blood	Oxygenated Blood
	cells/serum	
pH 7.1		
Chloride .....	.80	.77
Bicarbonate .....	.95	.90
pH 7.4		
Chloride .....	.73	.68
Bicarbonate .....	.89	.78
pH 7.7		
Chloride .....	.65	.60
Bicarbonate .....	.82	.66

\* Calculated from Dill, Edwards and Consolazio.<sup>18</sup>

the serum, not only in oxygenated and reduced blood, but also with varying pH.

**Acidosis and alkalosis.**—The extreme limits of pH of the blood plasma during life range from 6.9 on the acid side to 7.8 on the alkaline side. The  $[\text{HCO}_3^-]$  may vary from 10 to 100 vol. per cent (4.5-45 mM/l.), the  $\text{CO}_2$ -tension from 15 to 65 mm., and the  $[\text{CO}_2]$  from 0.5 to 2.1 mM. These values are, roughly, the outer limits of the chart in Figure 12.

The term *acidosis* is commonly used to denote ketosis, diminished alkali reserve (the height of the  $\text{CO}_2$  absorption curve), diminished  $[\text{HCO}_3^-]$ , diminished  $\text{CO}_2$ -tension or lowered pH of the plasma. *Alkalosis* is identified mainly with increased pH and  $[\text{HCO}_3^-]$ . Variations from the normal have been divided into four types by Peters and Van Slyke,<sup>56b</sup> and are represented as zones in Figure 12: (1) primary alkali deficit (metabolic acidosis), (2) primary alkali excess, (3) primary  $\text{CO}_2$  deficit, (4) primary  $\text{CO}_2$  excess. The term "alkali" as used in this description refers only to the cations neutralized by  $[\text{HCO}_3^-]$  or by other buffers. The usual type of acidosis seen clinically results either

from excess acid production or ingestion, or from loss of positive minerals. Alkalosis results when excess alkali is ingested or mineral anions are lost from the body. The secondary effects are those which result from compensation of the body, so that it is useful and informative to plot the paths by which these adjustments have been made, as has been done in Figure 12. Thus either compensated acidosis or alkalosis may result in a diminished  $[\text{HCO}_3^-]$ . The third and fourth types result from respiratory causes and are discussed in the next section.

Summarizing the careful studies on buffer values of blood, Peters and Van Slyke have shown that 80 per cent of the  $\text{HCO}_3^-$  in the blood may be neutralized in extreme acidosis. Of the buffers, per liter of blood, which counteract acid, the  $\text{HCO}_3^-$  may furnish 18 meq., hemoglobin 8, and the other buffers 2, of which the serum protein is 1.7. For the total circulating blood this is equivalent to about 150 cc. of 1.0N acid.

Van Slyke and Cullen<sup>75</sup> showed that when enough  $\text{H}_2\text{SO}_4$  was injected to account for the whole available blood alkali,  $\frac{5}{8}$  of the neutralization was accomplished outside the blood, so that the total alkali physiologically available in extreme acidosis is equivalent to 1 liter of 1.0N acid. They state further that the accuracy of such estimates is questionable; but they suggest that there is, in the average normal subject, enough available buffer to receive about 15 cc. of 1.0N acid per kg. of body weight before the blood or tissue pH falls to a fatal value.<sup>56b</sup>

#### EXCRETION OF ANIONS AND CATIONS

To maintain the normal acid-base equilibrium of the body it is essential that excess anions or cations be excreted. These products arise from the food ingested or from the metabolic processes of the body. The oxidation of carbon, sulfur and phosphorus in metabolism produces bicarbonate, sulfate and phosphate whenever tissues are destroyed. The incomplete oxidation of fats, as in diabetes, leads to the formation of  $\beta$ -hydroxybutyrate and other organic anions or free acids. Bacterial metabolism in the intestine usually leads to anion formation, although cations may be produced in excess. The retention of mineral cations in excess of mineral anions in growth may lead to mineral anion excretion. The main cause for excess cation excretion (other than  $\text{H}^+$ ) is the loss from the body of hydrochloric acid, by vomiting.

The excretion of alkali is carried out with greater facility than that of acid, and therefore more attention will be given to the latter. The regulation of acid-base equilibrium by excretion is carried out through three important physiological channels—lungs, kidneys and intestines.

## Lungs

The  $\text{HCO}_3^-$  is excreted by the lungs as  $\text{CO}_2$ , the anhydride of  $\text{H}_2\text{CO}_3$ . This is accomplished without loss of any mineral cation. By far the largest fraction of acid is removed from the body by this means; as much as 20-40 mols of  $\text{H}_2\text{CO}_3$  are excreted daily by the lungs.

The  $\text{HCO}_3^-$  of the blood plasma defends the body against excess acid. When part of the  $\text{HCO}_3^-$  is neutralized the pH of the blood would become more acid if the excess  $\text{CO}_2$  were not removed (see p. 278). The removal is controlled partly by the action of the respiratory center, which reduces the  $\text{CO}_2$  tension by hyperventilation. This hypernea or "Kussmaul breathing" is one of the cardinal signs of acidosis. It was formerly assumed that the diminished pH was the stimulant of the respiratory center, but Harkins and Hastings<sup>32</sup> have demonstrated that the mechanism is a compromise between the pH and the  $\text{CO}_2$  tension. About two-thirds of the effect is due to  $\text{CO}_2$  tension and one-third to pH. The degree of excitability is probably determined by conditions within the respiratory center.

The  $\text{CO}_2$  tension in the alveolar air which is in equilibrium with the arterial blood was formerly used as a measure of acidosis, but this has given way to the more accurate methods of blood gas analysis. Tensions of 35-53 mm. lie within normal range. Low values occur in either acidosis or hyperpnea, whether the latter is produced by hot baths, diminished atmospheric pressure, fever, or central irritation of the respiratory center. In order to determine whether this condition is compensated acidosis or alkalosis, apart from the clinical symptoms it is necessary to know the course that the acid-base equilibrium has followed. This is well shown in Figure 12, which emphasizes that there are, in the main, two types of respiratory disturbance, due to diminished or to excess  $\text{CO}_2$  tension.

When  $\text{CO}_2$  is removed from the blood, as by voluntary over-breathing, the ratio of  $[\text{HCO}_3^-]$  to  $[\text{CO}_2]$  is increased and alkalosis results. The excess cations are then removed from the blood and excreted in the urine. A compensation thus takes place, and the  $[\text{HCO}_3^-]$  is reduced until the pH approaches normal. As a result of such hyperventilation tetany may be induced in normal individuals. Forced ventilation brings on attacks in epileptics. When hydrochloric acid is lost, as from vomiting, the plasma  $[\text{HCO}_3^-]$  increases and may reach a value nearly double the original. The body compensates somewhat by excreting an alkaline urine, but when the  $[\text{Na}^+]$  of the plasma falls slightly below the normal level, this compensatory excretion of cations stops and the urine becomes acid, in spite of the alkalosis. Until chloride can be furnished either by mouth or parenterally alkalosis must continue, for the body cannot excrete cations further without lowering the

osmotic pressure of the blood. The body's only defense is to increase the  $\text{CO}_2$  tension. "Gastric tetany" may supervene.

The reverse of this condition may be seen in certain types of heart disease in which the alveolar tension of the  $\text{CO}_2$  is increased. In this case or in the case of breathing high concentrations of  $\text{CO}_2$ , a  $\text{CO}_2$ -acidosis results. This stimulates excess breathing in an attempt to return to normal.

### Kidneys

**Acid urine.**—The kidney is the main organ for the excretion of anions and cations. Urine is a buffer system with a pH usually about 6.0, although this may vary between 4.7 and 7.8. The ability to excrete urine either more acid or more alkaline than the blood permits the removal of either anions or cations when present in excess of normal. Chemically speaking a solution is acid when its pH is lower than 7.0. However, even urine with a pH of 7.0-7.4 represents excretion of acid because, although alkaline, it is less alkaline than the blood.

There is considerable confusion in the nomenclature of the factors which determine the acidity of the urine, and in the interpretation of their significance. These factors are mineral anions— $\text{Cl}^-$ ,  $\text{SO}_4^{--}$ ,  $\text{HPO}_4^{--}$  and  $\text{H}_2\text{PO}_4^-$ ; organic anions, including  $\text{HCO}_3^-$  and undissociated organic acids; mineral cations— $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ; and organic cations, chiefly  $\text{NH}_4^+$ . The usual amounts of these components and their roles in the excretion of an acid urine are now considered.

**"Free acid" or titratable acidity.**—The titration of the urine back to pH 7.4 as suggested by Henderson and Palmer<sup>36</sup> is the easiest and most convenient single measure of the acid excretion. This represents the buffer capacity of the urine between the two pH values, and has been called the "free" or titratable acidity.

Urine differs from blood in that the main buffer system which regulates acidity, especially on the acid side, consists of the phosphates. Their pK is 6.8 [see equation (3)]. At the pH of the blood (pH=7.4) 80 per cent of the phosphate is divalent ( $\text{HPO}_4^{--}$ ); the remaining 20 per cent is monovalent ( $\text{H}_2\text{PO}_4^-$ ). Hence  $(0.8 \times 2 = 1.6) + (0.2 \times 1 = 0.2) = 1.8$ . Therefore at this acidity each mol of phosphate is associated with 1.8 eq. of cations. At pH 4.7 the cation equivalent value per mol is about 1, and at pH 7.8, about 1.9. Thus, when the kidney excretes urine of maximum acidity, the phosphate is in the form of  $\text{H}_2\text{PO}_4^-$ , and for each mol of phosphate excreted 0.8 eq. of cation is retained by the blood, and an equivalent amount of anion, called "free acid," is excreted in the urine. This takes place according to the following reaction:





The amount of "free acid" depends upon the amount of phosphate and other buffers excreted and the pH of the urine. The pH characterizes the ratio of  $\text{HPO}_4^{--}$  to  $\text{H}_2\text{PO}_4^-$ , but the total phosphorus must be known to calculate the "free acid" excretion. When extra anions are to be excreted, the phosphate excretion is deflected from the feces to the urine, and thus a larger excretion of "free acid" is produced. Normally 10-40 meq. of anions (100-400 cc. of 0.1*N* acid) are thus excreted daily.

Although most of the organic acids are burned within the body, normally a small amount, and in diabetes a large amount of  $\beta$ -hydroxybutyrate is circulated in the blood to be excreted. As the dissociation constants of this and most other organic acids of physiological importance lie near 5.0 at pH 4.7-5.0, half or more may be excreted in acid form, although in the blood they are completely neutralized. As much as 1 eq. of undissociated acid (1 liter of 1.0*N* acid) may be excreted daily.

The pH of the urine characterizes, therefore, not only the ratio of  $\text{HPO}_4^{--}$  to  $\text{H}_2\text{PO}_4^-$ , but also the ratio of organic anions to organic acids. If organic acids are present the value for "free acid" includes them.

*Ammonium.*—The formation of  $\text{NH}_4^+$  by the kidney is one of the fundamental mechanisms of anion excretion. Excreted anions may be neutralized by  $\text{NH}_4^+$  and thus cause no reduction of the body supply of mineral cationogens. Ingested  $\text{H}_2\text{PO}_4^-$  is excreted as such and hence does not increase the  $\text{NH}_4^+$  in the urine.

Normally 30-40 meq. of  $\text{NH}_4^+$  are produced daily, and this accounts for about half of the anions in excess of mineral cations. When the urine is alkaline this may be diminished practically to zero and in acidosis astounding amounts, up to 500 meq. of  $\text{NH}_4^+$ , may be produced. In acidosis the excretion of  $\text{NH}_4^+$  may be so great as to lead to a diminution of  $[\text{Cl}^-]$  in the plasma. In nephritis the ability to form  $\text{NH}_4^+$  is impaired so that even in intense acidosis the  $\text{NH}_4^+$  may not be markedly increased.

The sum of the "free acid" and the  $\text{NH}_4^+$  in the urine has been called "total acid" and considered a measure of anion excretion, but this is inadequate, because it does not take into consideration the mineral cation excretion.

*Mineral cations.*—The positive minerals excreted in the urine have their origin either in the food ingested or in the body substance. Most of the positive minerals in the food are associated with negative minerals (p. 291). If the positive minerals are thus neutralized in the ingesta and also in the excreta, there is no net gain or loss to the body. When a neutral diet is consumed and the body is neither gaining nor losing minerals the excretion of mineral cations depends upon the cationogen content of the food. When food with acid- or alkaline-ash value is ingested or when acid is produced in the body, the situation is altered. Normally the body has the capacity, by extending or contract-

ing the "free acid" and  $\text{NH}_4^+$  excretion, to retain either mineral anions or mineral cations at need and to excrete the excess of either. Not only is this true when a neutral salt like sodium chloride is ingested, but, as will be shown later, the body may receive excess of mineral anions and retain mineral cations.

When the "free acid" excretion and the  $\text{NH}_4^+$  formation mechanisms are not adequate, the body may neutralize acid from its extra stores of positive minerals. When no food is taken, the mineral anions and cations in the urine come from depletion of body stores (see *Fasting*, p. 307).

The literature has not clearly differentiated between the excretion of organic and inorganic anions and cations, both of which are present in the urine. Excretion of organic cations permits the presence of mineral anions in the urine without loss of mineral cations; on the other hand elimination of organic anions is accompanied by loss of mineral cations.

Normally the  $\text{NH}_4^+$  in the urine equals about 30 meq., the mineral cations 148 meq., the mineral anions 140 meq., the organic acid 49 meq. and the "free acid" 23 meq. (see Table 11, p. 80). Roughly, the mineral cations and anions are equivalent, and also the organic acid and  $\text{NH}_4^+$ , and there is some "free acid" in addition.

*Effect of acid ingestion.*—The efficiency of the organism in withstanding acid ingestion is shown by the experiments of Haldane. He found that, in order to keep the urine of a normal adult continuously highly acid, the plasma  $[\text{HCO}_3^-]$  reduced and the pH below normal, it was necessary to give 20 gm. of  $\text{NH}_4\text{Cl}$  (370 meq.) per day, or 30 gm. of  $\text{CaCl}_2$ .<sup>56b</sup> Gamble, Blackfan and Hamilton<sup>23</sup> have demonstrated the acidifying effect of the neutral salts,  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$  and  $\text{MgSO}_4$  in metabolism. The  $\text{NH}_4^+$  of the former is converted to urea and the calcium and magnesium of the last two are excreted by the intestine, thus leaving an excess of mineral anions to be excreted in the urine. If given in sufficient amount they produce acidosis. These salts are used therapeutically in tetany, in which it is desirable to render the metabolism more acid.<sup>25, 26</sup> The magnitude of the effect of ingested acid upon the mineral cation excretion is deferred until later in the chapter.

**Alkaline urine.**—When an excess of cations is to be excreted the body reacts in several ways: (1) the urine may become alkaline, and more cations may be associated with the phosphates than at the pH of the plasma; (2) cations may be neutralized by organic acids; (3) cations may be excreted partly in association with  $\text{HCO}_3^-$  (in this way the body escapes depletion of mineral anions); and (4) mineral anions may be excreted (sulfates, phosphates and chloride). In actual experience the body is both more efficient in removing cations than anions, and less called upon to do so. In normal persons ingestion of about 4-5 gm. of sodium bicarbonate suffices to render the urine alkaline.

Alkalosis occurs in man primarily after loss of gastric juice, as in vomiting, or after administration of alkalis, or as a result of hyperventilation. Herbivora ordinarily consume an alkaline-ash diet and excrete an alkaline urine. In these animals silicate functions as an important additional anion available for the removal of cations.

### Intestines

The feces form a third means of anionogen and cationogen excretion. The physiologic needs of the body for the excretion of minerals are not covered completely by the kidneys, because only readily soluble materials can be taken care of by this means. The slightly soluble substances, especially calcium, magnesium, phosphates, carbonate and fatty acids, can be excreted in the urine to only a limited extent. The feces provide a means whereby they may be excreted in the solid phase. Thus it happens that the feces usually contain an excess of positive minerals. In diarrhea a considerable amount of cations may be excreted in the feces, resulting in what has been called by Steinitz<sup>71</sup> a "relative acidosis" (see pp. 86-89).

The mineral content of the food is the principal regulator of the paths of excretion, except when vitamin D and parathyroid hormone are involved. Acid ingestion is accompanied by increased urinary excretion of mineral anions, especially phosphates; alkali ingestion causes increased excretion of both calcium and phosphorus by the bowel. The intestines thus function in an entirely different way from the kidneys, but both together help to maintain the equilibrium of the body. The intestines have neither so large nor so extensible a mechanism for excretion of excesses of mineral anions or cations as have the kidneys, but the extent to which the intestines can function in the emergency excretion of positive or negative minerals has not been adequately studied.

### STORAGE AND DEPLETION

The chemical composition of the body is organized so as to form a tremendous reserve for the maintenance of the body neutrality. The first line of defense is the capacity to excrete materials with the maximum economy of mineral anions or cations. The second line of defense is the ability of the blood, because of its buffers, to resist change of pH after the addition of either acid or alkali. The third of these mechanisms enables those changes of the blood which do occur to be communicated to the rest of the body fluids so as to minimize the change. These mechanisms have already been discussed. The final defense lies in the body stores, which are depleted or augmented to aid in the neutralization or excretion of excess anionogens or cationogens.

The mineral stores of the body lie in the bones and body fluids, both

intracellular and extracellular. When conditions require it these three sources are drawn upon; the skeleton furnishes calcium, phosphate and carbonate, the cellular fluids furnish phosphate, sulfate and potassium, and the extracellular fluids furnish sodium, chloride and bicarbonate. Following periods of depletion, or when minerals are superabundant, they may be stored in these depots.

The bones form the main reserve of positive minerals. Ordinarily one thinks of the bones as rigid fixed structures, but it has been shown that the trabeculae can be increased in size and number to form a depot for calcium deposition or withdrawal.<sup>6</sup> The stores of positive minerals available are tremendous, and only after a long-continued withdrawal due to lactation, low-calcium diet, thyroid or parathyroid excess, or metabolic diseases, is osteoporosis likely to become evident.

In bone salts phosphate is trivalent; when it is transferred to the body fluids the valence becomes equivalent to 1.8; when it is excreted in acid urine the valence may be 1. When 1 mol of  $\text{PO}_4^{---}$  is changed to  $\text{H}_2\text{PO}_4^-$  it requires 2 less eq. of cations to neutralize it, and there are 2 mols of phosphate in  $\text{Ca}_3(\text{PO}_4)_2$ . One gm. of bone salts, which contain 13 per cent  $\text{CaCO}_3$  and 84 per cent  $\text{Ca}_3(\text{PO}_4)_2$  can furnish 13.4 meq. of cations, if all the phosphorus is excreted in the urine as pH 4.5, according to the following calculations:

$$\begin{array}{rcl} 0.84 \text{ gm. } \text{Ca}_3(\text{PO}_4)_2 & = 2.7 \text{ mM} \times 2 \times 2 & = 10.8 \text{ meq. of cations} \\ 0.13 \text{ gm. } \text{CaCO}_3 & = 1.3 \text{ mM} \times 2 & = 2.6 \text{ meq. of cations} \\ \hline & & 13.4 \text{ meq. of cations} \end{array}$$

As the total ash of the skeleton weighs 2250 gm., the total store of excess cationogens in the bones of an adult represents 30 eq. (or 300 liters of 0.1*N* solution).

The excess of positive minerals in the body fluids may be similarly calculated. As a large part of the body cationogens is associated with mineral anions it is only the excess of positive minerals which is available to neutralize additional anions. The principal source of non-mineral anions in the blood plasma and interstitial fluids is the  $\text{HCO}_3^-$ , which amounts to only 25 meq./l.; the cations not neutralized by  $\text{Cl}^-$  represent an equivalent amount. The excess mineral cations in the tissue fluids cannot be so well computed. Palmer and Van Slyke<sup>54</sup> have calculated that it is sufficient to consider that 70 per cent of the body is a fluid of the same composition as blood plasma. According to these data, the body fluids comprise 50 liters of a solution containing 50 meq./l. of excess positive minerals; the total excess is, at a maximum, 2.5 eq. Therefore the total positive mineral stores of the body which are the sum of excess in bone and body fluids, equal approximately 32.5 eq., of which the bones furnish about 92 per cent. To this amount must be added the possible  $\text{NH}_4^+$  production.

Obviously the whole of this amount is not available to neutralize acid excess, for the body cannot exist to the point of complete dissolution as did the ill-fated DeSauty.\* In acidosis of the most severe grade the  $[\text{HCO}_3^-]$  may be reduced to less than one-sixth its normal value. Further, the body fluids may be diminished so that 20 per cent of the whole body fluids are lost. Moreover, all the excess of positive minerals is not available at once. On page 283 it was stated that the injection at one time of 1 eq. of acid was the maximum the body could tolerate. This would neutralize only 3 per cent of the total store of positive minerals in the body. In chronic conditions a much larger portion of the cations can be withdrawn, but the maximum extent of depletion of total reserves has never been determined.

The body excretes or retains minerals according to its needs. Thus  $\text{Na}^+$  and  $\text{Cl}^-$  are not always excreted in equal amounts, even though ingested as sodium chloride. The body may retain  $\text{Na}^+$  and excrete  $\text{Cl}^-$ , as has been shown by Gamble *et al.*<sup>27</sup> It can also function in the opposite direction in diabetes where a chloride deficiency exists, as has been shown by Peters, with a retention of  $\text{Cl}^-$  and excretion of  $\text{Na}^+$ .

#### POSITIVE AND NEGATIVE MINERAL INTAKE

The relations between positive and negative minerals in the equilibrium of the body, in excretion, in storage and depletion have been discussed. Although the excretion may be varied to meet the metabolic needs, the body equilibrium is the resultant of intake and output. Therefore the ultimate factor in mineral anion-cation economy lies in the relation of positive to negative minerals in the intake. Ingestion of considerable mineral acid leads to acidosis or acid poisoning, and large amounts of alkali to alkalosis. Although such conditions usually arise only as a result of faulty metabolism or direct therapeutic or pharmacological procedures, it is worth while to examine closely the part played by mineral anionogen or cationogen excess in the food. Since Liebig's time it has been recognized that the ash of food is either acid or alkaline in character. So much has been written on the importance of this characteristic that an evaluation is desirable on the basis of evidence now available.

The acid or alkali value of a food in metabolism is quite different from the acidity as it exists in nature. The excess of mineral anions or cationogens is not defined by the true acidity—the pH. Milk has an acid pH but contains an excess of positive equivalents. The true acidity of body fluids is due to the mineral anions and cations in buffer systems containing  $\text{H}_2\text{CO}_3$ ,  $\text{HCO}_3^-$ , organic acids and anions, organic cations,  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{--}$ , etc. Comparable descriptions of the acid-base

\*See quotation on page v.

equilibrium of plant juices have been made. But when food is taken into the body and burned, the organic anions such as acetate, tartrate, malate, citrate, etc., are more or less completely oxidized. On the other hand, positive minerals in organic combination, such as the iron in ingested hemoglobin or the magnesium in chlorophyll, may be set free by digestion. Anions are formed by oxidation of the sulfur and phosphorus of proteins and by liberation of the phosphate of nucleic acids, etc. Because the acid value of the food in metabolism is so different from the true acidity of the food as ingested, the customary terms *acid food* or *alkaline food* are without specific connotation. In this book we have used instead the terms *acid-* or *alkaline-ash value of food*, or simply *acid-ash food* or *alkaline-ash food* to indicate the excess of negative or positive mineral equivalents calculated from analysis of the ash of the food.

### Critique of Food Analysis

**Methods of analysis.**—Early determinations of the mineral content of foods were made largely by agricultural chemists whose primary interest was in the total minerals removed from the soil. They analyzed the whole plant or the material "as purchased." The usual procedure was to analyze the ash after incineration, although it is now known that potassium, chlorine and sulfur are lost by this manipulation. Data on mineral content of biological material were expressed in oxides— $\text{Na}_2\text{O}$ ,  $\text{P}_2\text{O}_5$ , etc. This was done because it was thought that the sum of the parts would add up to the total of the ash, certainly a hopeless task when carbonates and pyro- and metaphosphates were present, and a worthless one if the ash did not represent all the elements present before incineration.

Kastle in 1908<sup>44</sup> proposed a method of incineration and titration of the ash of milk with phenolphthalein as an indicator. He stated that this method gave a value similar to those obtained by analysis and calculation. Approximately 15 meq./l. of excess mineral cationogens were found in both cow's milk and human milk by this procedure, although by Sherman's method cow's milk contains three or four times as much excess of positive minerals. It is worth while to point out that incineration of foods with an alkaline ash or an acid ash produces quite different types of compounds. This is because, as metaphosphate, the phosphorus has only one-third the combining value of orthophosphate, and because the amounts of ortho- pyro- and metaphosphate depend upon the ratio of cations to phosphorus. Therefore a procedure justified for milk might give erroneous values for meat. Although such a method has been readvocated<sup>22</sup> it has not received general recognition and should be abandoned.

In 1907 Sherman and Sinclair, and later Sherman and Gettler<sup>63</sup>

published a method of calculating the acid- or alkaline-ash value of the edible portion of foods according to their content of elements by analysis. Forbes,<sup>21</sup> Berg<sup>9</sup> and others<sup>59, 65</sup> have developed the method, now almost everywhere accepted, for calculation of the excess of positive or negative mineral equivalents of food "as purchased," "edible portion" and "ready to serve." The essential principle is the expression of the minerals in terms of chemical equivalents, that is, in meq. or in cc. of normal solution. This at once permits comparison of the various elements in common terms of chemical activity, and the excess of positive minerals (sodium, potassium, calcium and magnesium) or of negative minerals (phosphorus, sulfur and chlorine) may be easily calculated. This excess of potential mineral anions or cations is called the *acid-ash* or *alkaline-ash value* of the food.

Extensive tables of the mineral content of foods have been compiled. The most elaborate are those of Koenig, which are in part summarized by Albu and Neuberger.<sup>1</sup> Modern compilations in terms of meq. were introduced by Sherman<sup>62</sup> and elaborated by Berg.<sup>8</sup> For an example of the method, see Table 10 (p. 73), showing the calculation of the alkaline-ash value of milk.

Berg has included iron among the cations; and although this is theoretically correct, the quantities are so small that they are less than the probable error of the determination, and hence may be neglected. He also included  $\text{NH}_4^+$  and  $\text{N}_2\text{O}_5$  and calculated them as positive equivalents. This is clearly in error, because they may be either formed or destroyed in the body. Aluminum is classified by Berg as a cation. If it were excreted in the feces as phosphate it should be thus calculated, but this has not been observed in human beings.<sup>67</sup> If introduced as aluminate it would have anion, but not cation value. The most recent evidence is that aluminum is not absorbed by the body, and can therefore be omitted. The inclusion of manganese leads to similar difficulties. The other elements occurring in traces should also be omitted, for their computation is not on a firm basis, and their amounts are too small to affect the result, but give a false sense of accuracy which the method does not possess.

Forbes<sup>21</sup> has shown that the amount of silicate in the urine of cattle may be sufficient so that it must be taken into account for its anion value, but this does not apply to man.

It is curious that although this method of computation has become common in physiology and in metabolism studies, it has not been used at all where it should have been first applied, *i. e.*, in animal experiments. Here the use of purified diets has required the addition of minerals. It is customary to add a certain percentage of salt mixture to the diet, without any reference to its anion-cationogen value. Even in Osborne and Mendel's Salt Mixture XXX,<sup>53</sup> which was designed as a neutral

mixture, there is no indication of how its value was calculated, nor of how it is to be used with various amounts of protein, or with proteins containing different amounts of sulfur and phosphorus. This practice has obscured the effects of the acid- or alkaline-ash value of diets upon metabolism.

**Interpretation of data.**—While the method outlined above undoubtedly gives a correct foundation for an understanding of the acid- or alkaline-ash value of foods in metabolism, the resulting data must be applied with a knowledge of certain difficulties.

**Calculation of phosphorus and sulfur.**—The first difficulty comes in the basis for computation. Sodium, potassium and chloride are monovalent. Calcium, magnesium and sulfate are divalent, but what is the valence of phosphate? Sherman and Forbes have assigned to phosphate the valence of 2.0; Berg has used 3.0. This alters the calculated acid-ash value of meat about 60 per cent. At the pH of the body fluids, four parts of divalent and one of monovalent phosphate occur, and hence in the blood serum the combining value is 1.8 eq./mol of phosphate. When phosphorus is metabolized, it is practically all excreted as inorganic phosphate, but when it is stored part of it is in organic combinations. In the present calculation a tentative value of 80 per cent of the phosphorus of the body has been considered to be phosphate. But part of the organic phosphorus also has anion value, which has not been adequately determined. Because the mineral substrate of bone is similar to apatite— $n\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$ —the phosphate in bones has a valence of 3; this represents over 80 per cent of the body phosphorus. When excreted in the feces as  $\text{Ca}_3(\text{PO}_4)_2$  it is also trivalent. In the urine, at pH 4.5, the phosphate is monovalent, and the anion value increases with the alkalinity of the urine. It seems best to calculate the valence of phosphate as equivalent to 1.8, the condition in the body fluids, but this is merely a stoichiometric convention which must be modified to meet the needs of the problem being studied. (See also p. 21.)

Sulfur presents an additional difficulty. When it is excreted, a portion is found in the urine as "neutral" sulfur, which cannot be computed as having anion value. This portion of the sulfur was correctly estimated by Sherman and Gettler<sup>63</sup> and deducted from the total sulfur in the urine. When sulfur is stored, it is present primarily in protein, and as such has no anion value. It has been suggested that 25 per cent of the sulfur stored may be calculated as inorganic sulfate, but this tentative value is probably too high.

Whatever may be their anion value in storage, when the sulfur and phosphorus compounds in the body are broken down they are oxidized before excretion and hence the anions are increased just as though the anion intake were increased. This should be calculated in metabolic studies.



*Variability of mineral content.*—Foods, as all biological material, show variation. These differences are shown not only in the varying amounts of fat, protein and carbohydrate, but also in the minerals. Animal tissues are more constant than those of plants. The latter show differences according to the soil in which they are grown. Many recent studies have shown that alteration of calcium, phosphorus, nitrogen and potassium results from different acidities and mineral contents of the environment.

For gross studies the average values obtained from food tables have proved of great value, but in any metabolic experiment where the excreta are to be analyzed, a sample of the food used must be reserved for analysis. Food tables represent average analyses and rarely give, as do Atwater and Bryant,<sup>3</sup> the maximum and minimum as well. By glancing at the usual tables it would appear that the values are constants, and Berg, especially, has carried out his computation to include non-significant figures. In fact, a comparison of Sherman's and Berg's values show few agreements, many differing from 50 to 100 per cent. Therefore it must be again emphasized that in the present state of knowledge such data can be used only for orientation and rough approximation.<sup>48, 70</sup>

*Effect of different minerals.*—Knowledge of the excess of the positive or negative equivalents ingested gives no insight into the fractions that are present; the individual elements should not be overlooked. If the predominant cation is  $\text{Na}^+$  and the anion  $\text{Cl}^-$ , we recognize that the important feature may not be the neutrality, but the  $\text{NaCl}$  effect. Forbes' suggestion that a given excess of anionogens or cationogens may have a variable effect at different levels of salt intake is of importance and should be further investigated.

If the cation were calcium instead of sodium, it would at once become important to know the phosphorus intake, for the metabolism of each depends upon the ratio of  $\text{Ca/P}$ . There is also the additional problem of the effect of a given excess of positive or negative equivalents at a given  $\text{Ca/P}$  ratio.

If the cation were principally calcium and the anion were  $\text{Cl}^-$  or  $\text{CO}_3^{--}$  (instead of phosphorus) the problem would again be altered. For example, if  $\text{CaCO}_3$  is added, it has only cation value, for the  $\text{CO}_3^{--}$  is a non-mineral anion. Added  $\text{CaCl}_2$  has the same effect on acid-base equilibrium as if 75 per cent of the  $\text{Cl}^-$  were given as  $\text{HCl}$ <sup>9, 26</sup>; the  $\text{Cl}^-$  is excreted in the urine while the calcium is excreted principally by the bowel as calcium soap, calcium phosphate and calcium carbonate.  $\text{CaCl}_2$  or  $\text{CaSO}_4$  which, chemically speaking, are neutral salts, thus function as acidogens when metabolized, for they remove mineral cations from the body (see p. 287). It has been customary to calculate added  $\text{CaCl}_2$  on this basis, but not that in the diet. Similarly, if a mixture of

$\text{CaCO}_3$  and  $\text{NaCl}$  is added, should it be calculated as a slightly alkaline mixture of  $\text{CaCl}_2$  (which has excess anion value in metabolism) and  $\text{Na}_2\text{CO}_3$  (which has cation value only), or should this be computed as  $\text{CaCO}_3$  alone—hence only cations? This problem has never been raised in mineral metabolism studies.

*Other factors.*—The effect of food upon the body may be very different from that expected from the stoichiometric excess of mineral anions or cations. If the minerals are being stored, cations are stored in excess of anions. If minerals are removed from the body by breakdown of body tissue, the anions produced by the oxidation of sulfur and phosphorus compounds are in excess of the cations. Thus the acid- or alkaline-ash value of the diet may be altered in metabolism, depending upon whether storage or depletion is taking place.

Regardless of the acid- or alkaline-ash value of the food, if the caloric intake is too low, equilibrium cannot be maintained. Similarly, the protein intake may be too low to prevent body wastage, and high-fat diets are associated with organic acid formation and removal of positive minerals. Further, the vitamin content may be inadequate for proper assimilation or storage of minerals. Iron, iodine, copper and other traces in the food may so alter the metabolism of the body that food of the same acid- or alkaline-ash value, as calculated stoichiometrically, may lead to varying conditions of nutrition and health. Thus the results obtained in metabolic experiments must be ascribed to the acid or alkali effect only after other factors have been ruled out. The calculated acid- or alkaline-ash values are undoubtedly correct and their effect on metabolism clear when the differences are large, but when small there is considerable uncertainty as to their significance.

### Acid- or Alkaline-Ash Value of Food

*Desirability of acid- or alkaline-ash diets.*—Seventy-five years ago it was known that cereals, meats and eggs have acid ash and that milk, fruits and vegetables have alkaline ash. But what constitutes the optimal value of positive or negative mineral excess in foods is still an unsolved problem.

Certainly there are many who are loud in their enthusiasm for the merits of an alkaline-ash diet, especially Berg<sup>9</sup> in Germany, Hinhede<sup>41</sup> in Sweden, and Bischoff *et al.*<sup>10</sup> in the United States. In the main their claim rests upon two arguments: (1) because milk has an alkaline ash and is the food provided by nature for the use of the young of the higher animals it would seem *a priori* that an alkaline-ash food is desirable, especially for infants and children; (2) inasmuch as the body normally excretes an excess of mineral anions in the urine, acid-ash food places an extra strain upon the mechanisms of excretion; in Sansum's terminology it is like putting extra residue into a furnace already

clogged with ashes. In sickness the condition of acidosis is more frequent and more feared than alkalosis. In chronic and subacute conditions a long-standing difficulty in acid excretion may exist unrecognized. Hence it is claimed that an alkaline diet is both good therapy and good prophylaxis. Chittenden's prolonged study<sup>14</sup> demonstrated that athletes and soldiers fed low-nitrogen vegetable diets, presumably alkaline-ash, were capable of more than average sustained effort. However, Greenwald has written,<sup>30</sup> "Medical literature is rich in references to the supposed ill effects of an acid diet, but most of these will not stand a careful examination . . . most of the evidence indicates that the body is able to neutralize the excess of acid that may be formed by neutralizing it with ammonia, at the expense of the urea of the urine."

Berg has emphasized that, owing to the storage of sulfur and phosphorus in protein during growth, milk (or other food) is more alkaline for the infant than for the adult who burns these to phosphoric and sulfuric acids. He draws the probably erroneous conclusion that adults require a greater alkaline-ash value. But this neglects the obvious fact that the body stores cations in excess of anions in growth, especially in bones. Moreover, except under special conditions, adults require no excess cations for storage and excrete acid with greater facility than children.

If the acid-ash value of the diet were of major importance the effect should be reflected in the acid-base equilibrium of the blood. But Noorden<sup>52</sup> has succinctly summarized the data: "Everywhere, whether from north or south, east or west, from whatever countries, races or sects, whether the food comes from acidifying meat diets or acidifying meat, egg, cheese, cereal and legume diets, or from alkalizing milk, fruit, root and leafy vegetable diets, the same actual pH of the blood occurs."

A generation ago the main interest centered on the effect of acid- and alkaline-ash diets upon nitrogen metabolism because of the discovery of increased  $\text{NH}_4^+$  in the urine in diabetic acidosis. The mechanism of acidosis is now better understood, and because the effect of ingested acid or alkali upon nitrogen metabolism is minimal such studies are now seldom pursued.

No one has demonstrated either clinically or experimentally that the acid- or alkaline-ash value of a diet of natural foods is either beneficial or harmful. We are not even in a position to say whether milk neutralized with hydrochloric acid is better or worse for normal nutrition of infants than untreated milk. As far as milk made acid with organic acids is concerned, it is apart from the problem, for these acids are oxidized in the body.

It is not clear what part the acid- or alkaline-ash value of diets may play in the course of nutritional diseases. Much has been written to show that rickets is associated with acidosis and tetany with alka-

losis, but this factor is of only secondary importance. Diets high in fat produce  $\beta$ -hydroxybutyric acid, especially in children, and hence are acidifying. Such diets, and likewise starvation, which owe their effect to non-oxidizable organic anions rather than to excess mineral anions, have been used successfully in the treatment of epilepsy and pyelitis. Other studies have been made which show that alkali favors more efficient oxidative and immunological reactions, but not within the magnitude of changes that can be accomplished by diet.

Infancy, pregnancy and lactation present special problems. Infants, whose need of excess positive minerals for body growth is obvious, universally depend upon milk with its excess of cations. Women must furnish an excess of positive minerals for the formation of the fetus and the secretion of milk during pregnancy and lactation; hence they have special dietary requirements. In general it appears that alkaline-ash diets are those of election during growth.

Omnivorous animals, such as dogs and rats, can take larger amounts of HCl or  $\text{NaHCO}_3$  than can possibly occur in human dietaries and show no harmful effects after prolonged administration. Man also has defenses which can cope successfully with greater excesses of positive or negative minerals than occur in natural diets.

The benefit to health which so generally results from the use of diets consisting largely of fruits, vegetables and milk may be partly attributable to their alkaline residue. There are, however, several other ways in which liberal amounts of these foods are apt to be beneficial, notably by enriching the diet in calcium, phosphorus, iron and vitamins, and by improving intestinal conditions. Sherman<sup>62</sup> has expressed his opinion on the problem as follows: "The question whether or not there is merit under ordinary conditions in so choosing the food that the acid-forming elements thus introduced into the body shall be balanced by equivalent amounts of base-forming elements must, in the opinion of the writer, be regarded as still open at the present time [1936]."

There is no doubt that the adult in health can subsist upon diets that vary greatly in acid- or alkaline-ash value. Meat eaters and lacto-vegetarians both survive to recommend their type of diet. Eskimos and fruitarians both have offspring. Do we live longer, healthier lives because of the acid- or alkaline-ash value of the diet? Do we have more gout or kidney disease because of ingestion of excess negative minerals, or more arteriosclerosis and stone because of excess positive minerals? The problem can be solved only by statistical analysis of data on large numbers of cases.

**Acid- or alkaline-ash value of dietaries.**—It is possible to judge the importance of the acid- or alkaline-ash value of dietaries by making critical analyses of intakes which are considered to be satisfactory. This can be done in a variety of ways:

(1) The daily average individual food consumption can be calculated from data on the total food supply of a nation.

(2) The known food consumed by selected groups which have been studied can be subjected to similar calculation. In these two groups freedom of choice has usually been assumed, but it is obvious that this is altered by the food supply available, costs, food habits, prejudices, customs, religious observances and the extent of knowledge which determines choice.

(3) Animal experiments give data by analogy.

(4) Balance studies on isolated cases can be made.

*The food of nations.*—A survey of the food consumed in various parts of the world has been summarized by Greenwald.<sup>30</sup> When the acid- or alkaline-ash value of the food is estimated according to the method of Sherman and Gettler<sup>63</sup> the values for various nations may be summarized as follows, calculated in meq. of excess positive or negative minerals per man per day: (+ = positive; - = negative)

Russia	-19.5
United States	- 5.8
Great Britain	+ 1.5
France	+37.8
Germany	+48.2

These data show wide divergence from acid- to alkaline-ash values. The main determinant of the excess of positive or negative equivalents is the ratio of cereals to potatoes. In Russia and the Orient the large excess of cereals causes the dietaries to have an acid-ash value, as was true in the whole of Europe before the discovery of America. In the United States where meat furnishes such a large proportion of the protein, milk is consumed in smaller amounts, and hence the dietaries tend to have acid-ash value. The amount of fruit, vegetables and nuts eaten is too small to be a major factor. Further analysis of the food consumed in the United States is found in Chapter 14.

*Group studies.*—The material collected by Sherman in his study of the mineral intakes of 150 American families has been used for the calculation of the acid- or alkaline-ash value of the dietaries. These data are given in Table 26. It may be seen that, computed in this way, the most niggardly diets have acid-ash value, the most abundant, alkaline-ash, and the average have a slightly acid-ash. However, the excess of either comprises only 2-15 per cent of the total mineral content, and is least in the most abundant diets.

Studies by Blatherwick<sup>12</sup> of the diet of the American soldier in the World War indicate a variation of 39 meq. excess negative minerals to

Table 26.—Mineral Anionogen-Cationogen Value of American Diets.\*  
Calculated per person per day.

	Minimum (gm.) (meq.)		Maximum (gm.) (meq.)		Average (gm.) (meq.)	
Cationogens						
Na .....	0.19	8	4.61	200	1.94	84
K .....	1.43	37	6.54	168	3.39	87
Ca .....	0.24	12	1.87	94	0.73	37
Mg .....	0.14	12	0.67	56	0.34	28
Total <sup>1</sup> .....		+69		+518		+236
Anionogens						
Cl .....	0.88	25	5.83	164	2.83	80
P <sup>2</sup> .....	0.60	35	2.79	162	1.58	92
S .....	0.51	32	2.82	176	1.28	80
Total <sup>1</sup> .....		-92		-502		-252
Total minerals <sup>3</sup> .....		161		1020		488
Anionogen-cationogen value <sup>2</sup> .....		-23		+16		-16

\* Calculated from Sherman.<sup>20</sup>

<sup>1</sup> Cationogen values are indicated by +, anionogen values by -. The excess of anionogens or cationogens represents the acid- or alkaline-ash value of the food. This is a change in terminology from that formerly used by the author.

<sup>2</sup> The equivalent value of P is calculated as 1.8. No deduction has been made for organic P or S.

<sup>3</sup> The total mineral content is represented by the sum of the 7 elements. For discussion see Chapter 14, page 315.

24 meq. excess positive minerals, with an average acid-ash value of 2.2 meq./man/day.

*Animal experiments.*—Rats are indifferent to large changes of acid- and alkaline-ash value of intake. Gamble *et al.*<sup>24</sup> have shown that 5 per cent of the diet as  $\text{NH}_4\text{Cl}$  was not harmful to rats. McCollum<sup>49</sup> found no ill effect on growth and reproduction of rats fed exclusively on boiled egg-yolk, which is "probably the most acid of our naturally occurring foodstuffs." Lamb and Evvard<sup>46</sup> found no ill effects over three generations when 200-300 cc. 1.0N  $\text{H}_2\text{SO}_4$  were fed to swine, 5 cc. to rabbits, and 1.5-2.0 cc. to rats. Bischoff, Sansum and associates<sup>11</sup> reported no change in pH or  $\text{CO}_2$  of the blood whether rabbits were fed on barley (acid-ash) or alfalfa (alkaline-ash) over a period of two years. Long-continued acid feeding has an effect on the skeleton; the bones are depleted of minerals, especially calcium and carbonate.<sup>13, 28</sup>

*Analysis of individual diets.*—In complete mineral balance studies the analysis of the foods is essential. From such data one may easily calculate the acid- or alkaline-ash value of the intake.

Prisoners studied by Clark<sup>15</sup> were fed adequate mixed diets of two different types. The maximal individual variation (average for each diet period) was from 13 meq. of excess anions per man per day for

the high meat-milk diets (called "dry") to 32 meq. of excess cations for the meatless high-vegetable diets (called "green").

There is no report known to the author of deleterious effects of acid- or alkaline-ash diets in experiments with naturally occurring food-stuffs. Because the intakes are best considered in relation to retention and excretion, discussion is given in the next section, page 303.

*Summary.*—It is seen that the usual diets consumed by adults, calculated on the basis of positive and negative mineral equivalents, vary from acid- to alkaline-ash values, and, in the United States are nearly neutral. However, it should not be overlooked that the method of computation does not take into account the individual mineral components of the diet, and that the amount of each of these and their relation to each other must be considered. Further, the excess of positive or negative ionogens represents only a small percentage of the total mineral equivalents present. Diets which contain a preponderance of from 30 meq. of negative to 30 meq. of positive minerals per day, the body handles without apparent harm. The exact optimum value is impossible to define, but the best opinion favors slight alkalinity.

#### MINERAL CATIONOGEN-EXCESS BALANCE

In the chapter on body structure it was shown that the growth of the fetus and the infant requires a definitely calculable accumulation of minerals. It is now possible to compare the deductions made from that manner of approach with those reached by direct measurements of intake and output. Such material has been reviewed by Czerny and Keller<sup>17</sup> for infants, and by Shohl.<sup>64</sup>

Sherman and Gettler<sup>63</sup> showed that a shift from an alkaline-ash (potato) diet to an acid-ash (rice) diet produces an increase in urinary acidity. Salter *et al.*<sup>19, 58</sup> demonstrated further that the urinary acidity varies with the acid-ash value of the diet as calculated from analysis. But these studies and the many of which they are examples fail to take the feces into account. Such a procedure is justified in the study of the total mineral balance only when no feces are passed, *e. g.*, in fasting, and even in this condition minerals are excreted into and accumulate in the intestinal tract.

If the minerals of the food, urine, feces and sweat are all expressed in terms of equivalents, or in cc. of normal solution, a comparison of the intake and output can be made. It has been convenient in this discussion to summarize the effects of the various factors in urine and feces in terms of excess of mineral anionogens (P, S and Cl) or mineral cationogens (Na, K, Ca and Mg) excreted.

We have used the term *cationogen-excess balance* rather than the more cumbersome and less informative *mineral anionogen-cationogen*

*balance*, to replace the undesirable term *acid-base* or *base balance*. This is possible because the gains or losses of positive and negative minerals to the body can be expressed in terms of cationogen-excess alone, under one of the following categories:

1. Cationogen-excess in intake and anionogen-excess in output=positive cationogen-excess balance
2. Cationogen-excess in intake greater than that in output=positive cationogen-excess balance
3. Anionogen-excess in output greater than that in intake=positive cationogen-excess balance
4. Cationogen-excess in output greater than that in intake=negative cationogen-excess balance
5. Anionogen-excess in intake greater than that in output=negative cationogen-excess balance
6. Anionogen-excess in intake and cationogen-excess in output=negative cationogen-excess balance.

This concept is slightly different from that of the nitrogen balance or calcium balance, for it shows only whether the body is gaining or losing positive minerals *in excess* of negative minerals, or *vice versa*. Thus it is possible to have a negative cationogen balance, and at the same time a positive cationogen-excess balance (category 3) provided that the loss of anionogens is greater than that of cationogens.

The data from balance experiments may be summarized separately in four age groups: the breast-fed infant, the artificially fed infant, the child, and the adult. For discussion of the special conditions of fasting, see page 307, and of pregnancy and lactation, pages 342-4.

### Breast-fed Infant

The first mineral balance studies on breast-fed infants were made by Blauberg, and reported in 1900. About a dozen are now available.<sup>31, 43</sup> The data for the cationogen-excess balance from these experiments are summarized in Table 27. The average value of the milk consumed daily was approximately 10-15 meq. of cationogen-excess. (See also Table 10, p. 73.) The infants excreted a practically neutral urine and alkaline feces, and 3-5 meq. of positive minerals in excess of negative minerals were retained. This represents a positive cationogen-excess balance of approximately 0.5-1.5 meq./kg./day (category 2).

### Artificially Fed Infant

A number of studies are available concerning babies fed various milk mixtures. Shohl and Sato<sup>65</sup> and Swanson<sup>72</sup> have made such studies.



Table 27.—Mineral Cationogen-excess Balance of Infants.

	Breast-fed* meq. per day	Artificially fed †
Intake		
Cationogens‡	+24.9	+94.5
Anionogens‡	-14.7	-71.2
Excess (1)	+10.2	+23.3
Output		
Urine		
Cations	+6.9	+32.2
Anions	-7.1	-46.8
Excess (2)	-0.2	-14.5
Feces		
Cationogens	+9.2	+40.5
Anionogens	-1.9	-10.7
Excess (3)	+7.3	+29.8
Total		
Cationogen-excess (4)=(2)+(3)...	+7.1	+15.3
Cationogen-excess balance = (1)-(4) ..	positive 3.1	positive 8.0
Cationogen-excess balance per kg.....	positive 0.6	positive 1.0

\* Average values calculated from Czerny and Keller.<sup>17</sup>

† Average values calculated from Shohl and Sato.<sup>65</sup>

‡ Mineral cationogen values are indicated by +, anionogen values by -.

Cow's milk contains approximately four times the ash of breast milk, so that when it is modified by an equal volume of cereal and sugar diluent it still has a higher mineral content than woman's milk. These modified milk feedings give values for intake of the order of 20-30 meq./day of cationogen excess. The urine of infants given such feedings is definitely acid, and a large quantity of positive minerals is lost in the feces. The positive cationogen-excess balance is greater for these babies than for infants fed breast milk. (See Table 27.)

Experiments are available<sup>66</sup> in which infants were fed whole cow's milk, and also whole milk supplemented with extra salts of milk to make the total mineral content twice that of whole milk. In these cases retentions of all the minerals were increased roughly in proportion to the intake, but the cationogen-excess was not markedly increased.

No studies are available in which acid or alkali has been given to infants on breast feeding. Shohl and Sato<sup>65</sup> were able to show that when 25 meq. of acid were added to a modified milk feeding to make it neutral, the cationogen-excess balance was reduced only from 1.2 to 0.95 meq./kg./day. In a second case, with 47.3 meq. of  $\text{NaHCO}_3$  added, the cationogen-excess retention was increased from 0.8 to a value of 2.3 meq./kg./day.

## Child

Data on mineral balances of children are extremely rare except for calcium, phosphorus and nitrogen. Herbst's excellent study<sup>40</sup> is lacking data on sulfur metabolism. Sawyer, Baumann and Stevens<sup>60</sup> have studied two children aged 5 and 8 years, as shown in Table 28. These

Table 28.—Daily Cationogen-excess Balance of Children.\*

	Subject C. G.		Subject R. W.	
	Normal Diet	High Fat Diet	Normal Diet	High Fat Diet
Food † (1) .....	— 14.2	— 26.3	— 14.2	— 26.3
Output				
Urine				
Cations .....	+124.6	+149.4	+114.9	+144.2
Anions .....	—173.2	—191.1	—174.4	—200.8
Excess (2) .....	— 48.6	— 41.7	— 59.5	— 56.6
Feces				
Cationogens .....	+ 30.1	+ 45.5	+ 38.3	+ 37.8
Anionogens .....	— 18.7	— 28.2	— 26.7	— 21.9
Excess (3) .....	+ 11.4	+ 17.3	+ 11.6	+ 15.9
Total				
Anionogen-excess				
(4) = (2) + (3) ....	— 37.2	— 24.4	— 47.9	— 40.7
Cationogen-excess				
balance = (1) — (4) .	positive	negative	positive	positive
	23.0	1.9	33.7	14.4
Cationogen-excess				
balance per kg. ....	positive	negative	positive	positive
	1.0	0.08	1.45	0.6

\* Calculated from Sawyer, Baumann, and Stevens.<sup>60</sup>

† Mineral cationogens are indicated by +, anionogens by —.

data demonstrate an average retention of 1.2 meq./kg./day of cationogen-excess, a value practically the same as that found for infants. This is an example of category 3 (p. 301).

When considerable fat was substituted in the diet for an equal number of calories of carbohydrate, ketosis and acidosis were produced. The alkali reserve of the blood serum fell from 50 vol. per cent to 29 vol. per cent of total CO<sub>2</sub>. During the periods when the children were in a state of acidosis the cationogen-excess retention was reduced by half in one case, and in the second case completely eliminated (categories 3 and 5). It can be seen that the high-fat diet actually reduced the excess of negative mineral equivalents in the urine and increased

the excess of positive minerals in the feces. In spite of the increased acid-ash value of the food the anionogen-excess of the output was diminished, and therefore the cationogen-excess balance was diminished.

### Adult

The first complete mineral balance study of the adult was published by Wendt in 1905.<sup>77</sup> He determined the mineral balance of two subjects on a normal diet, with varying salt content, for four days. The data are complete for only two days. Unfortunately he did not determine sodium and potassium separately, and the values assigned to them here are approximated by calculating the average ratio of Na/K in the food as 3/1. The data would be more valuable if the subjects had been in equilibrium throughout the experiment and had not previously been on nitrogen- and salt-poor diets. The alkaline-ash value of the food was 2.7 meq./day of excess positive minerals. The subjects showed large positive cationogen-excess balances for the first and second days of 87 and 23 meq., or 1.3 and 0.35 meq./kg. of body weight.

The most elaborate and complete mineral balance study of adults is that by Clark<sup>15</sup> (see Table 39, p. 333). He fed analyzed diets to five prisoners (av. wt. 66 kg.). For the first period of 16 weeks the diet had an acid-ash value of 0.5-36.5 meq./man/day, and from the 17th to the 28th weeks, an alkaline-ash value of 4.8-68.5 meq. He calls the food the "dry" and "green" diets respectively. Each diet contained sufficient calories so that some weight increases occurred. The individual balances from week to week varied so that all categories of cationogen-excess balance from 1 to 6 are represented. In the long run all the men retained an excess of mineral cationogens over anionogens. During the period preceding the experiment they must have received an inadequate diet, for although only three of the five gained weight, all continued to retain considerable amounts of positive minerals during the seven months of the experiment.

The three individuals whose periods can be compared had positive cationogen-excess balances of 15.5 meq. on the acid-ash diets (category 3), and of 21.6 meq. on the alkaline-ash diets (categories 1 and 2), calculated per man per day, or 0.18 and 0.26 meq./kg./day. The total gain in weight of these three men was 16.5 lbs. for the period when they were fed acid-ash diets, compared to 10 lbs. for the alkaline-ash diets. This demonstrates that the body has the capacity to store both water and an excess of cationogens in spite of anionogen-excess of this magnitude in the intake. Moreover, the acid-ash diets were fed first in all cases, so that the difference between the two periods is masked, for presumably the depleted minerals and protein had already been in large part restored before the alkaline-ash diets were given. Therefore, although the gain in weight on the alkaline-ash diets was less than on the acid-

ash diets, the gain in cationogen-excess was greater. This was still more marked per unit of weight gained; on this basis the excess of positive minerals stored was twice that of the first period.

Bassett, Elden and McCann<sup>5</sup> have studied the mineral balances of two normal men, and the effect of acid and alkali ingestion. Inasmuch as sulfur balances were not included it is impossible to calculate accurately the cationogen-excess balances. In the normal period one man gained in weight and the other lost; the cationogen-excess retention is closely correlated with this variation. The order of magnitude of positive cationogen-excess balance during gain is approximately the same as that given by Clark. The alkaliogen used was potassium citrate, 8-10 gm./day, and the acidogen was  $\text{CaCl}_2$ , 10-13 gm./day. The former increased the cationogen-excess retention in spite of the fact that potassium salts are known to be diuretic rather than to increase cation retention. The latter caused negative cationogen-excess balances, although this action was complicated by the use of parathyroid hormone, which in itself has a dehydrating effect.

The careful metabolism studies of Loeb *et al.*<sup>47</sup> include the effect of  $\text{NH}_4\text{Cl}$  on mineral balances. When 224 mM of  $\text{NH}_4\text{Cl}$ /day were added to the diet for three days there was a weight loss of 0.8 kg., a lowered  $[\text{HCO}_3^-]$  in the serum and only a slight diuresis. (See Table 11, p. 80 and Figure 8, p. 81.) Unfortunately the amounts of sulfur in the food and feces were not given, so that the cationogen-excess balance cannot be calculated accurately, but inasmuch as the sulfate in the urine was not affected by the  $\text{NH}_4\text{Cl}$  addition it is assumed that the amount in the feces was also unchanged. There was a loss of potassium and retention of chloride, and increased excretion of calcium and phosphate represented a withdrawal from the bones. The acid intake thus resulted in a negative balance of mineral cations (68 meq.) a positive balance of mineral anions (20 meq.) and a net negative cationogen-excess balance (category 5).

Wiley, Wiley and Waller<sup>78</sup> have made similar determinations using 100 mM of  $\text{NH}_4\text{Cl}$  daily for three days, preceded and followed by suitable control periods. The periods of  $\text{NH}_4\text{Cl}$  administration were accompanied by loss of weight, and followed by gains in the after period which more than made up the loss. The amounts of phosphorus and sulfur ingested and excreted were not given, so that the cationogen-excess balance cannot be calculated, but with  $\text{NH}_4\text{Cl}$  administration there was chloride retention and negative balance of mineral cations, so that the resultant was either category 5 or 6. Similar data are reported by Følling<sup>20</sup> for  $\text{NH}_4\text{Cl}$ . There is some difference of opinion in the individual studies as to the time relations between the excretion of the chloride and mineral cations and the rise of  $\text{NH}_4^+$ , but in the main they are in excellent agreement. Most of these studies were made to throw light

upon the reactions of patients with kidney disease who have been shown to respond in a similar manner except for lessened  $\text{NH}_4^+$  production.

Complete electrolyte balance studies have been made on two diabetics.<sup>2</sup> The subjects were first under complete control with insulin and their metabolism was essentially normal. The withdrawal of insulin caused glycosuria in both cases and marked acidosis in one. The men were studied continuously during the fore period, the acidosis and recovery. They both showed excess cationogen retention (category 2) during the fore period and excess cationogen excretion (category 4) during the period of withdrawal, differing in degree only. The essential feature of this type of acidosis is a marked loss of water through urine. Each subject lost about 1 kg. of weight daily. Sodium and potassium losses were of the order of 50 and 90 meq./day, respectively. The chloride excretion was small (differing from that in  $\text{NH}_4\text{Cl}$  acidosis), presumably because of the large amount of the organic anion,  $\beta$ -hydroxybutyrate, in the urine; the  $\text{NH}_4^+$  was also excessive for the same reason. The negative balances of calcium, 50 meq., and of nitrogen were greater than those resulting from  $\text{NH}_4\text{Cl}$  ingestion. During the recovery period storage of these materials took place until the amounts depleted were restored. The  $\text{NH}_4^+$ , which had increased slowly during acidosis, remained high for some days of the recovery period.

In none of the above experiments was the sweat determined. Because it was not present in visible amounts does not mean that losses did not occur by this mechanism. Atchley, Loeb *et al.*<sup>2</sup> calculated that the excretion of minerals by this means was greater in acidosis than in normal metabolism. Such experiments of short duration suffer from two further defects; the ammonium mechanism may take from 2 to 5 days to reach its maximum, and the calcium which can be withdrawn from the bones is not immediately available, but is released over a protracted period of time. Longer-time experiments, to include a period after the initial adjustment to lessened body fluids has been made, are needed to demonstrate the effects of chronic acidosis.

### Summary

The essential features of these experiments are that, when subjects are in equilibrium, ingestion of diets with either acid- or alkaline-ash values up to 30 meq./day does not affect the cationogen-excess balance significantly. The small positive cationogen-excess balances found in normal subjects are presumably due to the losses of excess cations in sweat, which were not determined. When subjects are gaining weight they retain an excess of positive minerals whether the diets are either as acid or as alkaline as above. When acidogen or alkaliogen of the order of 100 meq. is given to normal men in addition to the food, excre-

tion of excess cationogens and weight loss occur with the former, and retention of excess cationogens and water with the latter.

### FASTING

The main reason for the discussion of starvation in this account is to show the tenacity with which the body guards its store of minerals when none are supplied, and to show the mechanisms involved in the depletion. Other aspects of the physiology of starvation are discussed by Benedict.<sup>7</sup> We shall cite only a single experiment to show how, under such stress, the various parts of the body release their quotas of minerals.

When an individual fasts he must break down the body tissue to provide for energy needs. The first source of material available is carbohydrate in the form of stored glycogen. This source is largely exhausted in a few days. As long as life continues large stores of protein and fat remain. When protein is destroyed large amounts of sulfur and phosphorus are burned to form sulfate and phosphate, and this results in an excess of mineral anions in the body. Fat cannot be burned completely without simultaneous combustion of carbohydrates. According to the thesis developed by Shaffer<sup>61</sup> it requires one molecule of dextrose to burn two molecules of fatty acid. When the carbohydrate stores are exhausted the only antiketogenic materials available are the products of breakdown of fats, which form glycerin, and the carbohydrate fraction formed in the breakdown of proteins. This is, however, inadequate for the complete combustion of the fats; and in fasting ketosis results. The  $\beta$ -hydroxybutyrate thus formed is a further source of anions which must be excreted. It also accumulates in the blood to the extent of about 10 mM./l.

The degree of acidosis, dehydration and hypotonicity are indicated by the following values attained in the plasma which are reached in a few days and remain nearly constant thereafter. The  $[\text{HCO}_3^-]$  and  $[\text{Cl}^-]$  are diminished; the former from the normal of 27 meq./l. to about 20 meq., and the latter, normally 103, drops as low as 91 meq./l. Thus the  $[\text{Cl}^-]$ , although the anion of strong acid, shows greater loss than the  $[\text{HCO}_3^-]$ . There is a slight but definite lowering of the  $[\text{Na}^+]$  of the plasma which accounts for a decrease of about 7 meq./l. in the total mineral cations.

The most obvious effect of fasting is the loss of body substance. Benedict's subject L., fasting for 31 days, lost 29 pounds. All the anions and cations in the urine over the whole period were determined and, as there were no stools, the values represent the total excretion. The daily average was 93 meq. of mineral anions and 50 meq. of mineral cations, or an anion excess of 43 meq. During the whole period 1327

meq. of excess anions were excreted from an alkaline blood. In spite of this tremendous loss, compared with the total content of the body shown in Chapter 2 this represents only 4.5 per cent of the mineral anionogens and 2.5 per cent of the total mineral cationogens in the body. A small amount of urea and about 10 meq. of  $\text{Cl}^-$  per week were found in the sweat collected in the clothing,—less than that found normally.

As the fast progressed there was a diminution, not only in the basal metabolism and in the nitrogen output, but also in the amounts of all the minerals excreted. In general this diminution was rapid in the first four or five days, and then became more gradual, but the rates for the different elements were not parallel. The data are not reproduced here, for in general the same conclusions can be drawn from the following experiments.

We owe to Gamble *et al.*<sup>27</sup> the theoretical explanation of how the various minerals come to be excreted in the urine. Their illuminating experiments on epileptic children, fasted for 15 and 10 days for therapeutic purposes, serve as a model of the manner in which studies in mineral metabolism can throw light upon physiological mechanisms.

The excretions of all the minerals in the urine were measured and from the known composition of extracellular and intracellular fluids the amount lost from each of these sources was calculated as shown in

Table 29.—Composition of Weight Loss.\*

	Period (3 days)				
	I	II	III	IV	V
Protein oxidized .....	133	100	83	72	64
Fat completely oxidized .....	232	176	145	125	112
Fat incompletely oxidized .....	63	99	74	55	43
Extracellular water lost .....	357	81	-60 †	-26	-28
Intracellular water lost					
Due to reduction of cell volume....	353	114	-36	8	26
Due to destruction of protoplasm....	477	360	298	257	230
Total weight loss ‡ .....	1615	930	504	490	447
Gm. protein ÷ gm. fat .....	0.45	0.36	0.38	0.40	0.41

\* Gamble, Ross and Tisdall<sup>27</sup>, p. 654, Table 4. Reproduced by permission of the *Journal of Biological Chemistry*. These data are from a 15-day fast by patient A. G. The losses are calculated from measurements of N, ketone acids (as  $\beta$ -hydroxybutyric), Na and K in the urine.

† The minus signs indicate a calculated gain in water.

‡ The sum of the calculated values for weight loss is 3986 gm. The weight loss directly measured was 3920 gm.

Table 29. Of the total body weight loss, 62 per cent represented water, and of the total water lost, 67 per cent came from cell destruction, 20 per cent from reduction in cell volume, and 13 per cent from extracellular water.

They calculated further that the body water lost contained 403 meq. of mineral cations. Of this the sodium and potassium represented 126 and 225 meq. respectively, the magnesium 51 and the calcium 80 meq. The cationogens claiming excretion were present in the fluid lost, except for 67 meq. of calcium which must have come from the bones.

From the sum of the measured phosphates, sulfate and chloride, taken as the total anions, they subtracted the titratable acid and  $\text{NH}_4^+$ . The value thus calculated for mineral cationogens agreed very closely with that determined by analysis (see Table 30). From this table it

Table 30.—Analysis of the Process of Acid Excretion.\*

cc. 0.1N

I. Fixed base excretion (indirect estimate)			
Acid excretion in terms of base bound at pH 7.4			
Organic acids; directly titrated	= 2,400	organic acids	
Phosphates; 2,955 mg. $\text{P} \div 3.1 \times 1.8$	= 1,715	$\text{H}_2\text{PO}_4$	
Sulfates; 1,669 mg. $\text{S} \div 3.2 \times 2$	= 1,045	$\text{H}_2\text{SO}_4$	
Chlorides; 2,942 mg. $\text{Cl} \div 3.5$	= 840	$\text{HCl}$	
	<hr/>		
	6,000	total acid excretion	
Titrateable acidity of urine	= 1,226	base economy	
	<hr/>		
	4,774	total base excretion	
Ammonia; 2,890 mg. $\text{NH}_3 \div 1.7$	= 1,701	ammonia production	
	<hr/>		
	3,073	fixed base excretion	
II. Fixed base excretion (direct measurement)			
Na, mg. $3,073 \div 2.3$	= 1,336		
K, mg. $5,320 \div 3.9$	= 1,362		
Ca, mg. $266 \times 2 \div 4.0$	= 133		
Mg, mg. $312 \times 2 \div 2.4$	= 260		
	<hr/>		
	3,091		

\* Gamble, Ross and Tisdall.<sup>27</sup>, p. 605, Table 8. Reproduced by permission of the *Journal of Biological Chemistry*. These data are from urine collected during the 4-day fast of the patient D. M.

can be seen that roughly one-half of the mineral anions were neutralized by mineral cations, and that the  $\text{NH}_4^+$  and titratable acid excretion were of almost equal amount. Of the mineral anions the phosphate was 48 per cent, the sulfate 30 per cent and the chloride 22 per cent. However, inasmuch as the phosphate excreted was in excess of that produced by muscle breakdown, some of the phosphorus (approximately one-fourth) together with the calcium must have come from the bones.

These mineral losses were due in part to the destruction of body tissues to supply energy and in part to the effect of the resulting acidosis. This work throws light not only upon fasting, but on the whole problem of the effect of acid on body physiology. Even when tissue



is not destroyed, an excess of anions claims its quota of mineral cations, which can be provided only from the same sources as in fasting, namely, the body fluids and the bones.

Gamble *et al.* were able to show that when sufficient carbohydrate was fed to remove the acid end products of fat metabolism, considerably less protein was broken down. They therefore interpret part of the breakdown of protein as a mechanism to prevent overwhelming ketosis. Under such conditions they found that the body could excrete a sufficient excess of anions to permit the cations furnished by the breakdown of the protein to be stored in the body, and rebuild the body fluids. Under these conditions although the ratio of N/S in the urine remained constant, the N/P increased to three times its former value, indicating that phosphorus was also stored. But no such storage of calcium took place. The sodium, potassium and magnesium in the urine were low in relation to the nitrogen, only one-third to one-fifth the values found in fasting. This represents restitution of body fluids.

To illustrate further that retention is selective, they demonstrated in another case that after a four-day fast and three days of carbohydrate feeding and two days of 4 gm. of NaCl/day, on the last two days the urine was found to contain much more  $\text{Cl}^-$  than  $\text{Na}^+$ . Approximately half of the  $\text{Cl}^-$  was excreted, but only 13 per cent of the  $\text{Na}^+$ , which indicates that the  $\text{Na}^+$  was retained to neutralize  $\text{HCO}_3^-$  to a greater extent than  $\text{Cl}^-$ .

These authors showed also that when a child was fed a normal diet following a 10-day fast, only after the 5th day of feeding did the excretion return to normal. During this period there was conservation of both positive and negative minerals.

This important investigation not only demonstrated the mechanism of mineral anion excretion in relation to mineral cations, the effect of acidosis on such excretion, the independent control of the two ions of a salt and the relation of protein destruction to ketosis, but laid the foundation for explaining the relation of mineral metabolism to water metabolism, and showed that the cations, except calcium, excreted in the urine, did not come from special depots, but could be evaluated, on the basis of the sodium and potassium, into fractions comprising the intracellular and extracellular body fluids.

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## Chapter 14

### Mineral Intakes, Balances and Requirements

Throughout the book we have attempted to show the relations of the minerals to life processes. Each of the minerals has been shown to have specific and varied functions and to constitute a definite portion of the body and organs. In the last analysis the performance of these functions depends upon the consumption, utilization and excretion of the individual elements. The requirement, retention, and paths of excretion for each constitute separate problems. The question arises as to how much of each of the minerals we obtain and what constitutes minimal, maximal and optimal intakes.

Except for sodium chloride, and in recent times, iodine, the intake is more or less fortuitous; the amounts of the elements that happen to be associated with natural foodstuffs determine the consumption. Hence the mineral intake is really preselected and predetermined. It is our purpose, not to define the amounts of the various foods which must be eaten, nor to prescribe a "balanced diet," but to discover the principles on which adequate diets must depend. The minerals in relation to the kinds and amounts of food usually eaten are first discussed. The adequacy of the average consumption is then evaluated in the light of experiments in which the intakes of the various elements were varied, and of balance experiments in which the fate of ingested minerals was studied.

#### MINERAL INTAKE

##### Forms of Minerals Ingested

Uncertainty regarding the relative merits of minerals from organic and inorganic sources extended over a long period. The experiments of feeding purified diets to animals early showed that when the diet was otherwise adequate, the mineral content was a limiting factor. When inorganic salts were supplied in adequate amounts and proper proportions, successful nutrition was obtained. This showed that organic forms of the minerals need not be supplied. It was formerly thought that organic iron was essential for building of blood. On the contrary, in the treatment of nutritional anemia, hemoglobin is a poorer source of iron than are inorganic salts (see Chapter 9, *Iron*). Even phosphorus, which is usually ingested in organic compounds, need not be so supplied

(see Chapter 7, *Phosphorus*). Except for sulfur, which must be supplied in native proteins or amino-acids (see Chapter 8, *Sulfur*), all the mineral elements can be utilized whether furnished as inorganic salts or as organic complexes. As a result of such experiments, it is obvious that if minerals in foodstuffs as usually consumed are not adequate, the amounts required can be furnished as inorganic additions to the diet.

### Mineral Content of Individual Foodstuffs

The large amount of accumulated data on the mineral content of foodstuffs has been collected and summarized in tables by Sherman<sup>83</sup> and by Berg.<sup>12</sup> The former expressed the values as elements per 100 gm., and per 100 Cal.; the latter as oxides per 100 gm., and as milliequivalents. The method of applying these data is similar to that used for the calculation of the acid- or alkaline-ash value of foods (Chapter 13). The individual minerals can be computed in terms of equivalents, and the sum of the individual elements gives the total mineral value. Because this expresses all the minerals in common terms, no other method of calculation of the total mineral content has as wide a basis for evaluating mineral intake.

These computations have been made for a few representative foods, and are shown in Table 31.

Table 31.—Mineral Content of Representative Foodstuffs.

	Minerals per 100 gm.* (meq.)	Minerals per 100 Cal.* (meq.)	Average serving † (gm.)	Minerals per average serving (meq.)
Apples .....	5	8	150	7
Beef, lean loin .....	47	19	100	47
Cucumber .....	10	57	50	5
Lettuce .....	19	100	50	10
Milk .....	20	30	220	44
Peanuts .....	76	14	15 nuts	17
Potatoes .....	25	30	150	37
Watermelon .....	5	17	300	15
Flour, white .....	25	7	100	25

\* Sum of the values of the seven minerals. Calculated from the food tables of Sherman<sup>83a</sup> and Berg.<sup>12</sup>

† From the food tables of Locke.<sup>60</sup>

Neither calculation per 100 gm. nor per 100 Cal. is a satisfactory method of computation, for the total intake is determined by caloric value, but that of individual foodstuffs by bulk. Thus the minerals in peanuts and meat, while high per 100 gm. are low per 100 Cal. Conversely, the succulent vegetables have the highest value per 100 Cal., but are low per 100 gm. and lower per average serving. Obviously the mineral intake is not related to the caloric value as such, because sugar,

etc., are mineral-free. The only actual relationship between minerals and calories comes in conditions of over- and under-nutrition. The minerals are much more closely related to specific food intakes. The important factor is not the concentration per unit of food, but how many units of that food are actually consumed. In studying actual total food intakes as a possible criterion for determining standards of mineral requirement, the logical means of expression is in mineral equivalents per man per day, or per unit of body weight.

### Mineral Content of Average Dietaries

Pearl has given an analysis of the food eaten in the United States. Greenwald has summarized this, and also data for other countries, and for the whole world.<sup>36</sup> For our present purpose the former will suffice. Analysis of this type assumes that, on the average, food consumed is sufficient for body needs, and whereas it may be below the optimum, it is certainly above the minimum. It is difficult to estimate whether all food is included, and how much is actually eaten, or fed to animals, or wasted.

Sodium chloride presents a special problem. The fact that salt used in cooking, pickling, etc., often is not eaten, makes this item difficult to compute. Ordinarily the intake is estimated at 8-15 gm. per man per day. This amount is so large in proportion to the total minerals in food, that a small error in calculation of grams of NaCl makes a large error in total mineral equivalents. Eight grams of NaCl represents about 135 meq. each of Na and Cl, or more than half the total of the other minerals contained in usual diets. Salt therefore has not been included in these calculations.

Greenwald's summary of the American diet shows, when considered in terms of fuel value, a consumption of 3400 Cal. per person per day distributed among the different classes of foods as follows:

Wheat supplies 26 per cent of the total calories, meat, fish and eggs 24, milk and dairy products 15, sugar 13, other grains 8, fats 4, potatoes 3, other vegetables 2, and nuts and fruits 3 per cent. This illustrates that people eat a mixture, of which cereals are the largest fraction in respect to calories and, with meat and milk, constitute three-fourths of the total. These proportions of the various foodstuffs do not vary widely among individuals, groups or nations. These figures should be compared to the mineral content of these same classes of foods, as shown in Table 32. The daily average American diet as here represented contains 236 meq. of anionogens and 228 meq. of cationogens, a total of 464 meq., or 13.5 meq./100 Cal. Cereals and carbohydrates furnish nearly one-half of the fuel value, but only one-sixth of the minerals;

Table 32.—Distribution of Minerals in Foods of the United States.\*

Food	Na	K	Ca	Mg	Cl	P †	S	Total
	grams per person per day							
Meat, fish, eggs.....	0.211	0.847	0.029	0.059	0.189	0.539	0.573	
Milk .....	0.415	1.230	1.000	0.096	0.850	0.760	0.250	
Wheat + other grains	0.165	0.318	0.055	0.049	0.204	0.253	0.490	
Potatoes + other veg.	0.144	1.270	0.102	0.092	0.155	0.190	0.096	
Nuts, fruits .....	0.024	0.245	0.020	0.019	0.026	0.075	0.037	
Total .....	0.960	3.910	1.206	0.315	1.420	1.820	1.450	
	milliequivalents per person per day							
Meat, fish, eggs .....	9.2	21.8	1.5	4.9	5.3	31.0	35.8	109.5
Milk .....	18.0	31.6	50.0	8.0	24.0	44.0	15.6	191.2
Wheat + other grains	7.2	8.1	2.7	4.0	5.8	14.7	30.0	72.5
Potatoes + other veg.	6.3	32.6	5.1	7.6	4.4	11.0	6.0	73.0
Nuts, fruits .....	1.1	6.9	1.0	1.6	0.7	4.4	2.3	18.0
Total .....	41.8	101.0	60.3	26.1	40.2	105.1	89.7	464.2
	per cent of total minerals							
Meat, fish, eggs .....	22.0	21.6	2.4	19.0	13.3	29.7	39.6	23.5
Milk .....	43.2	31.4	83.0	30.0	59.8	41.7	17.3	41.4
Wheat + other grains	17.2	8.2	4.6	16.0	14.2	14.0	34.0	15.5
Potatoes + other veg.	15.1	32.5	8.4	29.0	10.9	10.5	6.6	15.7
Nuts, fruits .....	2.5	6.3	1.6	6.0	1.8	4.1	2.5	3.9

\* Calculated from Greenwald.<sup>38</sup>

† The equivalent value of P has been taken as 1.8 (see p. 293). The values are computed from the total P, without differentiation of its forms.

and milk, which provides only one-sixth of the calories, accounts for nearly half of the minerals.

It is interesting to compare the usual intakes of people with the mineral content of the diet of normal laboratory rats. As a measure of adequacy McCollum proposed the thesis that the diet should not only permit survival, but that it should also allow a normal span of life and activity, and the production of normal offspring for successive generations.

The most satisfactory state of nutrition has been attained by the use of a salt mixture comparable to the ash of cow's milk, which is usually fed at a level of about 4 per cent, by weight, of the dry diet. Adequate nutrition can, however, be attained with less than half this amount.

Calculation shows that the classical salt mixtures furnish minerals, at the 4 per cent level, amounting to about 60 meq./100 gm. of diet, or 15 meq./100 Cal., in addition to that inherent in the food. These values are comparable to those found in human diets. They include sodium chloride, while the calculations for man do not. Therefore they are lower per 100 Cal. than the actual intake of human beings. But small animals consume more calories per unit of body weight, and calculated on this basis, the rat diets contain three or four times as many mineral equivalents per kilogram of body weight as do those of human beings, even with sodium chloride included in the latter.

The total mineral content is a very poor index of the adequacy of the minerals in any given diet, for it has been our thesis that individual elements, or rather, certain groups of elements, perform separate functions in the body economy, and are therefore more important than the total.



When we calculate (from Sherman's food tables) the minerals derived from the given amounts of food, we obtain an insight, not only into the amounts of the individual elements consumed, but also the sources from which they are obtained. Table 32 shows the quantity and distribution of the individual minerals expressed in various ways. It is interesting that, on the basis of weight, the potassium comes first, and the calcium on which so much stress is laid, is the lowest, except for magnesium. When considered as chemical equivalents, the phosphorus, even calculated at a valence of only 1.8, has the highest value, and again calcium is the lowest, except for magnesium (sodium chloride not considered).

A cursory inspection is sufficient to reveal that at the levels of intake given, milk furnishes not only the preponderance of the total minerals, but also of each of them individually (except sulfur). Its greatest contribution is calcium, of which it supplies four-fifths of the total. It leads the list with regard to magnesium, sodium, phosphorus and chlorine, and contains as much of the potassium as the combined vegetables. In sulfur it is surpassed by both the meat and the wheat consumed. It is thus readily appreciated how well founded are the general claims of the importance of milk in the dietary, with respect to minerals, and to what quantitative extent milk is a protective food. It is possible to construct milk-free diets by expansion of the amounts of nuts, fruits and green vegetables, which are adequate in most respects, but the calcium still remains a problem. With meat-free diets none of the minerals becomes restricted except the quantity and quality of sulfur, and this is obvious from the success obtained with lacto-vegetarian diets. To obtain sodium- and chloride-poor diets it is necessary to reduce the proportion of milk.

The total minerals ingested by the 150 families studied by Sherman have already been given in Table 26, (p. 299). These average values do not differ markedly from those obtained by the above analysis, except in regard to the calcium content. It seems to the reviewer that the amount of milk intake in Greenwald's data is more liberal than the usual consumption, and that therefore the calcium values represent a desirable amount and those given by Sherman more nearly the actual consumption. Sherman's data also have the virtue of showing minimum and maximum values, and so indicate that the diets of many individuals are far below the average.

By actual weights and measurements of food intakes and wastage, Murlin and Hildebrandt<sup>71</sup> have shown that the food consumed by American soldiers in training closely approximated the distribution given above except that slightly more meat and less cereal were consumed. The Tigerstedts<sup>102, 103</sup> analyzed the food consumption of groups in Finland and reported a comparatively large consumption of milk. Studies by Hornemann<sup>46</sup> in Germany, and also studies of groups in

England correspond closely to those of Sherman. These data, as those cited for the United States, do not include added sodium chloride.

Many studies have been made of the food intakes of children, and are excellently reviewed in the White House Conference<sup>112</sup> and by Czerny and Keller.<sup>23</sup> Most of these have been made from the point of view of energy metabolism, or of calcium, phosphorus and nitrogen metabolism, and none, even including the excellent study of Herbst<sup>42</sup> gives exact information concerning all seven minerals. The interesting study by Davis<sup>24</sup> in which infants from below one year of age were allowed to make free selection of their diet from a large variety of natural foods, shows that these infants chose amounts of protein, fat and carbohydrate quite comparable to our dietary standards, and that nearly half of the calories consumed were from milk, as is customary with ordinary infants. The high milk intakes of infants and children provide large amounts of minerals compared to adult diets.

A discussion of optimal intakes and calculated requirements as compared to average intakes is given under *Mineral Requirements*, page 352.

### High and Low Mineral Intakes

The study of low and high mineral intakes is important for at least the following two reasons: first, by means of deficiency and excess the safe variations may be determined; secondly, when the body is put under stress, light is cast upon physiological mechanisms. The close relationship between levels of mineral intake and water metabolism has been discussed in Chapter 12, *Water Metabolism*, and in Chapter 3. Inasmuch as normal human dietaries are concerned, with naturally occurring food-stuffs, the problems of high and low mineral intakes have to be studied under very special conditions. This question has not been sufficiently studied in man for general discussion. The problem is best set forth by the more extensive animal experimentation.

The important low-salt studies of the pre-vitamin era have been repeated with vitamin additions. The studies at Yale<sup>91, 114</sup> have definitely proved that a sufficiently low mineral content of the food prevents growth of rats. With casein (high-phosphorus) diets anemia supervenes, but does not when edestin furnishes the protein.<sup>97</sup> Additions of a "complete" salt mixture at a level of 0.5 per cent will protect or cure such animals.

In conformity with the findings of McCollum, Bing\* found that moderately increased salt mixtures were without observable harmful effects upon rats. He has further shown that at a level of 16 per cent of the total weight of the diet, a "complete" salt mixture will prevent gain in weight and, at 32 per cent, will cause death.

\* Dr. F. C. Bing, personal communication.

The work of Meyer<sup>68</sup> and of Schloss<sup>81</sup> demonstrated beyond question the importance of the minerals in the diet of infants. When the milk whey was removed from the food, as much as 15 per cent of the body weight was lost within a week, but was regained rapidly when the whey was re-introduced. It is unnecessary to cite other data on feeding with various combinations of casein and fat, with and without whey, which support this rather obvious conclusion. It should be realized, however, that the diet may be qualitatively defective in any single element. (See also under intake of individual minerals.)

Comparable experiments on adult human beings are not available. Taylor<sup>99</sup> experimented with a diet of adequate caloric value consisting of washed egg-white, washed fat and sugar, and containing only 1 gm. of ash per day. This resulted in a weight loss of 1.5 kg. in 9 days, and immediate recovery after discontinuance of the experiment. Although he remained in nitrogen equilibrium, the urine became practically free of chloride. Goodall and Joslin<sup>84</sup> confirmed these results except that the weight losses were even greater. These results must be interpreted as a readjustment of interstitial fluid volume at different levels of mineral intake. Presumably the organic content of the cells was little affected because nitrogen equilibrium was maintained.

Wendt's studies<sup>110</sup> of low-salt intake with various additions of salts and different levels of protein intake well merit repetition with less frequent changes. Such regimes show the acid products of protein destruction as do starvation experiments. Inadequate protein diets cause destruction of tissue protein and their contained salts, and high protein-low salt diets conserve the body protein but result in negative cation-oxygen-excess balance.

## BALANCE STUDIES

### Critique

The measurement of intake and excretion and the calculation of the resulting balance whether positive, negative, or zero, determines whether the body is storing material, losing it, or is in equilibrium. Much valuable information has been collected by this excellent method, beginning with nitrogen, and extended to all the minerals. Such studies do not yield knowledge of how this material is distributed. They do not disclose, for example, whether iron is retained in the liver or blood, or in what form phosphorus is stored or withdrawn. Therefore this method can never give more than a bank balance—it cannot state how the income was invested but only how much was spent. It serves then as a gross check on any further interpretation of the intermediary metabolism. However, when the bodily state is known to be normal, or its abnormality is appraised, far-reaching deductions can be made as to

the source of material excreted or the site of material stored from what is known of the composition of the body.

In general the plan of metabolism studies has been to observe the balances of individual elements when these are varied in the diet, and to use negative balances as a criterion of insufficiency, and positive balances, of superfluity. Adults who are neither gaining nor losing weight should remain in mineral equilibrium. The amount of the mineral just sufficient to bring this about represents the requirement of that individual for the element under investigation. Unfortunately, as for most biological data, this amount cannot be given with great precision whether in terms of body weight or surface area. The value at best can be only an average one based on statistical analysis. When used in this way it has considerable scientific validity. It furnishes a standard which can be applied to groups, but gives no information as to the individual case. Leitch<sup>50</sup> has analyzed the data of 400 metabolism experiments and shown that, except for the most extreme levels of intake, there are some positive and some negative balances on the same intake. She has considered that intake adequate which results in an equal number of cases of small positive and negative balances. This seems to be the best criterion at present.

When one applies this method to a study of conditions during which accretion is taking place, such as growth of children and pregnancy, the problem becomes much more complicated. The difficulty is to determine what retentions are optimal. Neither maximal nor minimal retentions should be so regarded. There is no ideal standard. Results can be compared only with adequate data obtained under known conditions. Rational empiricism is our best guide.

It is necessary to emphasize here again that mineral metabolism cannot be dissociated and studied apart from the rest of the body. Much of the data from balance experiments is so fragmentary and represents such poorly planned experiments that interpretation is difficult, and the results are often misleading.

**Methods.**—When balance experiments are made to measure the intake in the food against the output in urine and feces it is necessary that all three be analyzed by approved methods of known accuracy. The average analyses of constituents given in food tables do not suffice for balance studies in individual cases. The variation in the foods used may be as much as 20-50 per cent from the expected values. Because of the unknown constituents of foods, the actual foods consumed must be recorded, not simply their mineral content.

It is necessary to know the probable error of determinations made, and the standard deviation, in order to determine whether altered intakes give values whose differences are significant.

If the intake of water is not constant the amount ingested should

be recorded, as should the water content of urine and feces. When water balances are desired, temperature, humidity and activity must be known, and for accurate values the perspiration and insensible water loss must also be measured.

Too much reliance must not be placed on short-time experiments. There should be a suitable pre-period under observation, for mineral equilibrium is often attained gradually. Experimental periods less than seven days in length for the most part represent an insufficient duration. If less time elapses there is a further difficulty in allocating the feces to their proper period. Moreover, there is a variation in retentions of one individual from one period to another. Macy feels that conclusions cannot be drawn from less than four successive 5-day periods. The period of experimental intake should be followed by an adequate after-period. In the case of vitamin D studies, the after-period must be at least three weeks.

**Evaluation of data.**—The following criteria are offered as essentials in interpreting metabolism data.

**Intake.**—The components of the diet must be evaluated both quantitatively and qualitatively in regard not only to the caloric requirements, but also to the amount and character of the proteins, fats, carbohydrates and especially the vitamins.

It must be determined whether the minerals not being studied are present either in such scarcity or such abundance as to cause alteration in the total metabolism. In making such calculation the importance of the individual elements, including the "traces" and their relation to each other, must not be overlooked or neglected. A few milligrams of iron or a few micrograms of iodine may make the difference between nutritional success and failure. Further, it must be known whether the total minerals in the diet constitute a high or low level of intake. From these data the acid- or alkaline-ash value of the intake can be evaluated.

The water metabolism has recently been shown to be so intimately related to the minerals that distributions in retention should be known and evaluated for their interrelationship with the mineral metabolism.

When investigations of single elements are made one is unaware of simultaneous changes in the metabolism of other elements and the body's reactions in other respects. The more aspects from which the study is made the greater is the value of the data obtained. The remote effects, or as may be equally valuable, the lack of such effects, may be thus demonstrated.

**The subject.**—It is necessary to know and evaluate the age, weight, race and sex of the subject. Moreover, such points as the ratio of height to weight, type of body build, endocrine type, type of activity, may be significant.

A factor which must not be overlooked in such studies is the previous diet and condition of the individual. Many of the infants and children selected for study are not normal, and cannot therefore be utilized for the study of normal achievement. Too often they are in the hospital recovering from acute or chronic diseases. Some have been prematurely born, some have had rickets, and many have not grown normally from birth. Previous losses are often repaired with extreme rapidity, whereas former rapid gains may cause a standstill even in the presence of plenty. Hence the growth, which is the summation of many factors, does not proceed in a smooth curve. The factor of previous diet and condition of the individual applies with equal force to adults.

The balance of any given mineral varies from week to week in the same individual, even on the same diet.<sup>50, 62</sup> Further, various individuals on the same diet retain different amounts.<sup>48</sup> This must represent a different individual characteristic and also the result of the previous dietary history with regard to superabundance, adequacy or paucity.

The relation of the minerals metabolized to growth, and to weight gains or losses, must be critically analyzed. (See further on p. 329.)

### Normal Mineral Retentions

In order to gain an insight into the nature and extent of mineral balances it seems best to examine the studies made upon persons at various ages. In this way approximate standards for normal intake and retentions are disclosed. Later the proportions excreted by the various paths are described. The factors which affect the retentions and paths of excretion can then be discussed, and as a result the requirements for normal growth, development and maintenance can be proposed.

**Infant.**—The first complete studies of mineral balance were made by Blauberg<sup>14, 15</sup> under Rubner's direction. He simply stated his data and made little attempt at explanation. Since then hundreds of balance studies have been made upon infants, especially by the Breslau group, and a few upon children. An excellent summary and discussion of this work may be found in Czerny and Keller's monograph.<sup>23</sup>

**New-born infant.**—During the first fourteen days of life the infant, for practical reasons, is usually under the care of the obstetrician and only later comes to the pediatrician. It is during this period that physiological losses of weight occur. During the same time the character of the food is changing from colostrum to breast milk. The metabolic processes are then undergoing alteration. The meconium is eliminated and the digestion begins to function. It is during this period that the acidosis of the new-born, described fully by Ylppö,<sup>116</sup> occurs. Jaundice of the new-born also introduces problems of interest. The permeability of the intestine to proteins and immune bodies takes place. For all

these reasons this period forms a study apart from that of infants over two weeks old.

Czerny and Keller have devoted considerable attention to this subject from both practical and physiological aspects. In regard to the mineral metabolism during this interesting period we have studies by Michel, Langstein and Niemann, and especially by Birk.<sup>13</sup> The latter has made balance experiments on new-born infants fed colostrum, breast milk, and cow's milk modifications. He analyzed the food and excreta for ash, calcium, magnesium, sodium, potassium and phosphorus. Owing to the complicated problems involved and doubt as to the significance of very small positive and negative balances, none of the data is here reproduced.

*Premature infant.*—Just as the new-born child presents a problem by itself, so the child born prematurely differs from the normal infant. From studies of the fetus it was found that mineral deposition takes place most rapidly in the last three, and more especially in the last two, months of gestation. The premature child is born with deficits which must be made up, so needs extra minerals. He thus has an increased burden to carry with a double difficulty of a poor mechanism and a small capacity. It is not surprising that these babies fail. Their rapid growth and small intakes make them especially susceptible to rickets and anemia. Without treatment all of these babies show these two diseases, probably due to deficient intakes of calcium, phosphorus and iron. Breast milk from wet nurses who have received irradiated ergosterol, and to which iron has been added, has been shown recently by Gerstenberger to produce babies free from either rickets or anemia. The problem is then not one of mineral intake only, but also of vitamin D or irradiation. That vitamin additions will prevent rickets is proved, but whether the bone will be of normal composition remains a problem to be solved in the future.

A careful study of the intakes and outputs of premature infants was made by Hamilton.<sup>37</sup> He showed that the amount of calcium in the feces was of the order of that of normal breast-fed infants, and therefore because of low intakes the retentions were so small that the calcium stores of the body became depleted as the infant grew. Only at the age of four months or over did calcium retentions approximate those of normal babies. Phosphorus retentions, on the other hand, were greater than those of calcium, but were below normal for the first two months.

In a later paper he has extended his observations to include all the mineral elements, and has shown that only in sodium and chloride does the premature exceed the retentions of the normal full-term infant.<sup>38</sup>

It is generally accepted that prematurely born children do not reach normal standards until several years of age.

*Breast-fed infant.*—The data on normal children fed breast milk are given in Table 33. These comprise average values for seven experiments

summarized by Czerny and Keller. The values have been recalculated in terms of grams and equivalents of elements, instead of oxides. It is felt that these average values give a dependable quantitative insight into the intakes and retentions of normal breast-fed babies.

Table 33.—Mineral Balance of Breast-fed Infants.\*

	Average per infant per day					
	Na	K	Ca	Mg	Cl	P
	(mg.)					
Intake .....	119	209	220	40	255	129
Output						
Urine .....	43	133	14	11	182	34
Feces .....	20	27	126	16	13	26
Total .....	63	160	140	27	195	60
Balance .....	56	49	80	13	60	69
	(meq.)					
Intake .....	5.2	5.4	11.0	3.3	7.2	7.5
Output						
Urine .....	1.9	3.4	0.7	0.9	5.1	2.0
Feces .....	0.9	0.7	6.3	1.3	0.4	1.5
Total .....	2.8	4.1	7.0	2.2	5.5	3.5
Balance .....	2.4	1.3	4.0	1.1	1.7	4.0
	(% of intake)					
Balance .....	47	23	36	33	24	54

\* Calculated from Czerny and Keller,<sup>23</sup> p. 700 Table 264a.

Average weight of infants = 4.9 kg.

Average age of infants = 3 months.

Sulfur values are lacking.

These retentions vary from 13 mg. per baby per day for magnesium to 80 mg. for calcium, in the following increasing order: Mg, K, Na, Cl, P and Ca. Expressed as chemical equivalents the order is: Mg, K, Cl, Na, P and Ca. These data give the order of magnitude of the mineral gains per day of young, healthy infants consuming human milk and studied for short periods of time. The percentage retention varies from 23 per cent for potassium to 54 per cent for phosphorus. The former is probably furnished in excess of the needs, and the latter probably is barely sufficient to meet requirements. When considered in terms of equivalents it is seen that the sodium retained is sufficient to balance all the chloride and to leave enough excess to balance the bicarbonate which is formed; calcium and phosphorus are present in proper proportion to form bone and there is enough phosphorus extra for the requirements of cells and muscles.

We have recalculated these retentions per kg. of body weight, per 100 gm. gain in weight, by age and by increasing order of gains in



retentions. They have been correlated with both the retentions and the intakes per gm. of phosphorus, calcium, ash and nitrogen. Different individuals show closer similarity in nitrogen retention than in any of the mineral elements. The mineral retentions of the individuals are more nearly proportional to weight than to age, and even more closely related to intakes. The correlation between gain in weight and retention of minerals is especially poor. Inasmuch as the milk is constant in composition the intake of minerals is proportional to the volume of milk consumed. Bigger babies retain more not because they weigh more, but because they eat more.

The improvement in the nutrition of breast-fed babies when cod-liver oil is supplied is strikingly demonstrated in Swanson's study of a baby kept on breast milk from 1 to 3 months of age, and then given cod-liver oil in addition from 3 to 5 months.<sup>98</sup> The improvement in retentions is almost wholly confined to the calcium and phosphorus. The data are summarized in Table 34. However, the fact that his

Table 34.—Mineral Retentions of Breast-fed and Artificially Fed Infants.\*

	Breast Milk		Cow's Milk	
	Without Cod-liver Oil <sup>1</sup>	With Cod-liver Oil <sup>2</sup>	Without Cod-liver Oil <sup>1</sup>	With Cod-liver Oil <sup>3</sup>
	(meq./day)			
Sodium .....	2.53	2.24	4.87	5.62
Potassium .....	2.50	2.42	4.56	5.19
Calcium .....	0.20	3.58	5.74	14.56
Magnesium .....	0.40	0.74	1.64	1.26
Chlorine .....	2.02	2.00	4.84	6.08
Phosphorus <sup>4</sup> .....	1.46	2.66	5.35	8.05
Sulfur .....	1.12	0.68	1.00	0.08
Nitrogen .....	40.40	36.70	66.66	65.90

\* From Table 3 of Swanson.<sup>98</sup>

<sup>1</sup> Age of infant 2 weeks, 3 months. Eleven 6-day periods.

<sup>2</sup> Age of infant, 3-5 months. Two 6-day periods.

<sup>3</sup> Age of infant, 3-6 months. Four 6-day periods.

<sup>4</sup> Equivalent value taken as 1.8.

subject, after cod-liver oil, did not exceed the values in Table 33 indicates that the baby must have been markedly depleted in vitamin D stores at the beginning of the experiment.

*Artificially fed infant.*—Methods of infant feeding have changed greatly in the last two generations. Formerly very dilute milk mixtures with added carbohydrate were used in an attempt to simulate breast milk. The modern tendency has been to use increasing proportions of milk, and also higher caloric intakes. Since such feedings with the additions of vitamins C and D have been used very generally, the growth standards of the previous generation for both height and weight

have been consistently exceeded. We now know that growth may be limited either by caloric insufficiency, protein insufficiency, or lack of development of the skeleton. The modern baby is not only taller and heavier, but because of the lessened proportion of carbohydrate, is also less fat. It is not surprising, then, to find smaller mineral retentions in infants fed diets which frequently resulted in rickets, scurvy and anemia, than in those fed more generous diets.

The mineral content of modified milk mixtures is greater in nearly all respects than that of human milk, and the retentions are also greater. But there is no question but that a larger proportion of the minerals ingested in breast milk are retained than is the case with cow's milk feedings. It has been shown that with various types of modified milk feedings the retentions vary, and that they increase as the consumption of minerals increases, but not in direct proportion.<sup>98, 115</sup> Because mineral retentions are greater on cow's milk than on breast milk it is not implied that artificial feeding is superior to breast feeding in every respect, even though the superiority of the latter is less apparent than it was a generation ago.

The advancement in the understanding of principles of infant feeding is further dramatically attested by the successful use in infant feeding of foods which contain no milk. Tso<sup>107</sup> prepared a soy-bean "milk" which produced successful nutrition throughout the period of infancy when suitable additions of Ca, P and NaCl were made. (See also Stearns.<sup>93</sup>) These infants grew normally, and showed adequate retentions of Ca, P and N, by balance experiments. Such diets are used in this country only when infants are allergic to cow's milk, but are of tremendous practical importance in a country like China which does not produce milk.

Considerable data have been amassed from balance experiments on artificially fed infants. Wendt, in the second edition of his review,<sup>111</sup> states that a great deal of the material that had been included formerly

Table 35.—Daily Mineral Balance of Artificially Fed Infant.\*

Element	Intake		Excretion				Retention	
	(mg.)	(meq.)	Urine		Feces		(mg.)	(meq.)
Sodium .....	422	18	300	13	78	3	43	2
Potassium .....	1182	30	785	20	104	3	293	7
Calcium .....	1031	52	17	1	734	37	280	14
Magnesium .....	123	10	18	1	60	5	45	4
Chlorine .....	788	22	651	18	13	1	123	3
Phosphorus .....	804	42	457	26	210	10	137	8
Sulfur .....	400	28	241	13	13	1	146	9

\* From Shohl and Sato.<sup>87</sup> The subject was 8 months old, weighed 8.8 kg., had recently recovered from an acute infection. The intake was 833 cc. of cow's milk + 56 gm. of cane sugar + 229 cc. of water, equivalent to 85 Cal./kg.

must now be considered valueless because the vitamin content of the food had not been considered. This is undoubtedly true, and much of the early work can be accepted only after very critical reading of the original data.

For this reason, instead of giving average values, it seems best to cite a single example. The data given in Table 35 are presented not as a standard, but to illustrate the order of magnitude of the intakes, paths of excretion and retentions of an artificially fed infant. See also the data given in Table 34, and Table 41 (p. 337).

As a measure of the variations which are encountered the data of Wendt given in Table 36 will suffice.

Table 36.—Variations in Mineral Retentions of Artificially Fed Infants.\*

Element	Total Retention (mg.)	Retention per kg. of Body Weight (mg.)
Sodium .....	Neg.-185	Neg.-60
Potassium .....	105-446	23-84
Calcium .....	49-657	15-86
Magnesium .....	10-158	1-24
Chlorine .....	62-393	12-69
Phosphorus .....	41-436	6-66

\* Wendt.<sup>111</sup>

Data on calcium and phosphorus metabolism of infants are relatively common. Macy has collected over 400 such studies from the literature to 1933, and Leitch<sup>59</sup> has analyzed nearly as many. Unfortunately most of these studies were made without the addition of vitamins. A summary of Macy's data (unpublished) follows. The calcium intakes varied from 103 to 150 mg./kg./day, the phosphorus from 90 to 123 mg./kg./day. The range of retentions of calcium was 220-279 mg./day, or 30-50 mg./kg. from birth to the 2nd month, and 30-44 mg./kg. from the 2nd month to the 10 month. The range of retentions of phosphorus was 134-183 mg./day, or 18-32 mg./kg. up to the 10th month.

The excellent studies of the Iowa group comprise now over 400 balance experiments on the retentions of calcium, phosphorus and nitrogen. The infants studied were in the first year of life. They were fed whole milk plus 6 per cent carbohydrate, and adequate vitamin D supplements. The data show that, with an average intake of 135 mg. of Ca/kg./day, and 110 mg. of P, babies have retentions of the order of 50 mg. of Ca/kg. and 30 mg. of P. They report retentions as high as 60-80 mg./kg. of Ca, and 40-50 of P. Their average value is in conformity with Leitch's standard for retention of normal infants. These values are at least 50 per cent greater than those given by Sherman, or the usual values reported, or found by the author (Ca=35-40 mg./kg., and P=15-17).<sup>87</sup>

They exceed those which were given in Chapter 2 (p. 50) based upon analysis of the skeleton.

There are several ways of regarding this mass of data. One is that the experiments have been done in such number that they are valid statistically. Another is that they represent such a mixture of material that more reliance can be placed on fewer experiments, more carefully selected and controlled as to vitamin and mineral intake.

*Factors affecting retentions.*—Increased retentions with high intakes are obtained not only with natural foodstuffs, but also when the minerals are further increased by the addition of inorganic salts. In short-time experiments a baby was fed minerals at a level of twice that of whole milk (eight times that of breast milk). He retained minerals in almost the same proportion as when fed whole milk, and in nearly double the amounts. Whether this would have continued indefinitely is not known.<sup>88</sup>

Most of the experiments on mineral metabolism have been of very short duration. The finding of larger retentions on cow's milk has been interpreted either as "supermineralization" or as temporary storage. But the data of Swanson given in Table 34 show conclusively that this increased retention on cow's milk extends without interruption for at least a hundred days.

One need only cite again the data of Swanson to show in what different groups the same baby falls with and without vitamin D. So, too, Jeans, Stearns *et al.*<sup>52</sup> have found that although a few babies without added vitamin varied from low to excellent retentions of Ca and P, those which received 400 units of vitamin D showed consistently larger retentions and less variability. Without alteration in the food intake, the retentions of Ca and P are approximately 10 mg./kg. higher in infants when 300-400 units (International or U.S.P.) of vitamin D are given daily than when the dose is one-third of this amount.<sup>53</sup> For the artificially fed as for the breast-fed infant, the cod-liver oil effect is confined to retentions of Ca and P only (see Table 34).

The story has altered considerably in the last decade. Calcium and phosphorus retentions of 35-50 mg./kg. exceed the previous achievements. Such babies not only do not have rickets, but even exceed their antecedents in rate of growth both for weight and height.

*Interpretation.*—The earlier reviewers<sup>23, 111</sup> came to the discouraging conclusion that they were unable to correlate the mineral retentions with growth, composition of the body, phosphorus or nitrogen retentions, or the relations of elements to one another. The interpretation of this material and the understanding of the problem of mineral metabolism of infants has been clouded by the assumption that the retentions should be fixed and constant, because of the presumable constancy in body composition. Further difficulty has arisen from the belief that the addi-

tions due to growth should exactly resemble the composition of the body. If these theses were correct, the mineral metabolism would correspond to growth, irrespective of the type of feeding.

Our viewpoint is that neither of these assumptions is correct. It is necessary to divide growth as measured by weight increase into the several systems—skeleton, cellular tissues, interstitial fluids and fat. These are interrelated, but divergent. There seems little doubt that weight increase can occur in spite of deficits of Ca and P. But skeletal deficiency permits growth to only a certain extent. Greater retention permits a more rapid growth not only of the skeleton, but of the soft parts also. The cellular tissues require, in addition to protein, their quota of K and P, and the interstitial fluids increase mainly by the retention of Na and Cl. Weight increase may be due to fat alone, which is laid down practically mineral-free. Of these systems, the one most likely to show deficiency is the skeleton, because of inadequate supplies of Ca and P.

Table 37.—Calcium Intake and Retention of Children of Various Ages.\*

Age (Yrs.)	No. of Cases	Intake/kg./day		Retention/kg./day	
		Mean (mg.)	Range † (mg.)	Mean (mg.)	Range † (mg.)
1	13	117	70-164	27	7-47
3	16	70	58-82	23	7-39
4	32	52	35-71	12	6-18
5	38	52	31-73	9	0-18
6	34	50	41-59	11	6-16
7	15	43	31-55	9	0-18
8	26	51	27-75	12	4-20
9	21	47	31-63	10	0-20
10	58	42	23-61	7	1-13
11	46	36	19-53	12	5-19
12	30	28	15-41	7	1-13
13	18	32	19-45	5	-7-17
14	3	22	15-29	6	3-9
15	9	14	6-22	2	-2-8
16	32	12	1-23	-0.2	-4-4
17	15	13	5-21	1	-2-4

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\* Compilation of data from the literature by Dr. I. G. Macy (unpublished). Pathological material was not included, nor cases with intentionally high or low intakes.

† Standard deviation. This includes the middle  $\frac{2}{3}$  of the cases retained.

**Child.**—The difficulty with appraisal of the mineral metabolism of infants is that there are too many conflicting data; in regard to children, the difficulty is that there are too few studies to form a sure judgment. The only complete study available is that of Sawyer, Bauman and Stevens. Their data have been reviewed previously with regard to cationogen-excess balances (p. 303). As far as the individual elements

are concerned, there are so many negative balances in the "normal" periods, that the data are certainly not appropriate to use as normal standards. Macy and her collaborators have made many continuous balance studies on children, not only for all seven of the elements in question, but also for iron. Only part of the data on Ca and P have as yet been reported and these only in preliminary form.

With regard to calcium and phosphorus there is no scarcity of data. Moreover, the vitamin intake has not been shown to affect materially the metabolism of calcium and phosphorus in children and adults. Therefore the older data are not subject to the same difficulty of interpretation as was found for the infant. Reviews of this material have been given by Sherman and Hawley,<sup>55</sup> Czerny and Keller,<sup>23</sup> Schmidt

Table 38.—Phosphorus Intake and Retention of Children of Various Ages.\*

Age (Yrs.)	No. of Cases	Intake/kg./day		Retention/kg./day	
		Mean (mg.)	Range † (mg.)	Mean (mg.)	Range † (mg.)
3	16	79	61-97	23	0-37
4	30	59	46-72	11	5-17
5	26	59	34-84	8	-19-35
6	19	53	43-63	7	1-13
7	10	36	38-54	5	0-10
8	19	54	22-86	7	-6-20
9	15	55	31-79	6	1-11
10	41	46	26-66	4	-2-10
11	43	46	29-63	11	3-19
12	27	30	20-40	3	0-6
13	8	32	25-39	6	-3-15
14	3	37	24-50	11	5-17
16	5	45	34-56	10	4-16
17	6	35	28-42	3	2-4

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\* Compilation of data from the literature by Dr. I. G. Macy (unpublished). Pathological material was not included, nor cases with intentionally high or low intakes.

† Standard deviation. This includes the middle  $\frac{2}{3}$  of the cases retained.

and Greenberg<sup>52</sup> and by Leitch.<sup>59</sup> The original sources may be found therein.

The compilations in Tables 37 and 38 of the data on calcium and phosphorus metabolism from the literature are given through the kindness of Dr. Macy. The tables show in a very convincing manner that on the usual dietary intakes, calcium and phosphorus retentions continue throughout the period of growth. The retentions per kilogram of body weight diminish year by year until maturity, when equilibrium is reached. The total retentions of both Ca and P of children up to 12 years equal or exceed those of infants and young children, due to the

increasing weight of the child. From 8 to 12 years they represent a daily retention of 200-300 mg. of Ca per day, with a Ca/P retention ratio of 1.5/1. During late childhood the total amounts of Ca retained per day diminish and therefore the amount per kg. becomes still smaller. The total P retentions remain high. During this period the ratio of Ca/P in retentions become 1/1 or less.

Leitch has selected from the literature the maximal retentions. These values equal, for the 8- to 12-year old children, 300-500 mg. of Ca per day, and for the older group 450-650 mg. We consider that this procedure is not justified, as these data do not represent normal performance, and should not serve as a standard. They represent either unusually high intakes or repair from previous shortage or both. She selected these high values as a basis for her proposed standard which we discussed in Chapter 2 (p. 49), and concluded was too high.

It has been shown with children, as with infants, that retentions are proportional to the intakes. Sherman and Hawley<sup>85</sup> have demonstrated in a series of studies that the same child will retain greater amounts but a smaller proportion of the intake of Ca and P when these are increased in the diet by augmenting the quantity of milk. This holds good for amounts all the way from 250 to 1500 cc. of milk per day.

The remarkable ability of a child with malnutrition to store Ca, P and N is amply demonstrated in the study by Stearns and Moore.<sup>94</sup> This 3½-year old child, in a period of recovery from chronic diarrhea, doubled his weight in a period of 9 months. Three balance studies made at intervals showed that he was able to retain Ca and P at a rate of 60-70 mg./kg. of each, values which would be high for the most rapidly growing infant. Hunscher *et al.*<sup>47</sup> have reported that when two children of the same age and weight, one with a good nutritional history and the other with previous low mineral intake, were placed on identical diets, the latter showed Ca retentions three times as great as the former. Wang<sup>108, 109</sup> has also shown that undernourished children have a larger Ca and P storage, per kilogram, than normal children. However, x-ray studies of such children may show different degrees of physiological maturity, and therefore different responses may be due to anatomical rather than nutritional causes.

Darrow\* found that a baby during acute illness retained but five per cent of the Ca intake. On a similar diet after recovery he retained the astonishing amount of 95 per cent of the intake. That such different metabolic patterns are maintained for a very long period of time by individuals with Ca deficiencies is attested by the studies of Macy *et al.* on children.

The discussion of the intakes of calcium and phosphorus necessary

\* Dr. D. C. Darrow, personal communication.

to attain normal retentions and the factors which affect them favorably or unfavorably is deferred until p. 346.

**Adult.**—Presumably the adult of stationary weight is in equilibrium with regard to intake and output of minerals. However, none of the experimental data gives such values, so all must be subject to interpretation. A number of balance experiments were detailed in Chapter 13, under *Cationogen-excess Balance*.

By far the most complete study is that of Clark,<sup>19</sup> for description of which see page 304. A summary of his data is given in Table 39.

Table 39.—Mineral Balance of Normal Men.\*

	Average per man per day					
	Cationogens			Anionogens		
		(gm.)	(meq.)		(gm.)	(meq.†)
Intake	Na	3.94	171	Cl	5.17	146
	K	2.47	63	P	1.48	86
	Ca	0.88	44	S	0.93	58
	Mg	0.26	22			
	Total		300	Total		290
Output Urine	Na	2.30	100	Cl	3.90	110
	K	1.80	46	P	0.70	41
	Ca	0.09	5	(Sulfate S	0.53	33)
	Mg	0.09	8	Total S	0.61	38
	Total		159			189
Feces	Na	0.12	5	Cl	0.09	3
	K	0.47	12	P	0.51	30
	Ca	0.64	32	S	0.13	8
	Mg	0.20	17			
	Total		66			41
Total	Na	2.42	105	Cl	3.99	113
	K	2.27	58	P	1.21	71
	Ca	0.73	37	S	0.74	46
	Mg	0.29	25			
	Total		225			230
Calculated Retention	Na	1.52	66	Cl	1.18	33
	K	0.20	5	P	0.27	15
	Ca	0.15	7	S	0.19	12
	Mg	-0.03	-3			
	Total		75			60
	Cationogen-excess		15			

\* Calculated from the data of Clark<sup>19</sup> on the intake and output of 5 normal men for a total of 112 weekly periods. No volumes of urine are given.

† The P is calculated as 1.8 eq./M, the S as 2.0. These are mere stoichiometric conventions. In the urine the equivalent value of P is about 1.2, in the feces 3.0; in retention it is 3.0 in bone and undetermined in tissue. The value of sulfur in retention approaches zero when S is stored as protein.



When added salt is subtracted from the intake it is seen that Clark's values are more nearly comparable to Sherman's average intakes (Table 26) than to the U.S. standards (given in Table 32), which appear too generous. The experimental diets were slightly low in magnesium and sulfur only.

The diets used by Clark resulted in considerable retentions. At first thought it seems reasonable to conclude that these positive balances are commensurate with the weight gains. However, when the sodium and chloride balances are critically evaluated it is obvious that the calculated balances do not measure retentions of these elements. The Na and Cl above that excreted in the urine and feces were sufficient to account for an increase of 2 kg. of interstitial fluid per week, or the approximate equivalent of whole body weight for the period of the experiment. The discrepancy must be due to the loss by sweat. This is true not only for Clark's subjects, but also for the hospitalized individual of Loeb and associates (see Table 11, p. 80). He also consumed a large amount of Na and Cl not found in the urine and feces. This apparent retention was greater on normal salt intake than on low salt intake, which it followed, and may represent in part a repair after previous depletion. Similar results were obtained on two diabetics controlled by insulin, studied by Atchley, Loeb *et al.*<sup>6</sup> On low NaCl intakes both Bassett, Elden and McCann<sup>9</sup> and Wiley, Wiley and Waller<sup>113</sup> found negative balances of Na and Cl. Positive balances appear larger than they should, and negative balances appear less negative if the sweat is not considered. Atchley *et al.* thought that the amount as well as the composition of the sweat varied.

The sulfur and potassium retentions found by Clark were large and in such proportion to the nitrogen retentions as to indicate that these minerals were stored in proportions to form cellular proteins and cellular fluid.

The positive calcium balances were large throughout the whole experimental period, and of the order of those found in growing children. This shows, as Clark has stated, a long-standing inadequacy of the previous diets. Whereas these men retained calcium on a diet which contained 0.88 gm. of calcium per day, the man studied by Loeb was continuously in negative calcium balance with an intake of 0.44 gm. The diabetics mentioned above showed both positive and negative balances with average slightly positive, with more than one gram of calcium intake. The calcium retentions of Clark's subjects were larger on the alkaline than on the acid diets.

The magnesium balances which were negative are discussed on page 167.

The phosphorus retentions were considerable, and larger than necessary for combination with the retained calcium to form bone. The

excess must have been stored in cellular fluid, together with potassium (see Chapter 2). The phosphorus retentions were slightly greater on the acid-ash diets than on the alkaline-ash ones. Therefore there was a difference between the Ca/P ratio of retentions in the two periods. But in both cases the ratio (in grams) of the Ca/P stored was less than 1/1.

Stearns<sup>92</sup> has indicated that for infants with normal growth the ratio of Ca/P retained lies between 1.5/1 and 2/1, and that for growing children, because of the greater muscular development, it is lower, but still greater than 1 until puberty. The ratio for bone is about 2.15/1. Therefore the retentions of Clark's subjects during the entire experimental period must be interpreted as supplying materials for both bone formation and muscle increase, with the latter predominating.

Following Gamble's thesis,<sup>32</sup> it is possible to assess from mineral balance studies the gains and losses of the several body systems. In order to do this sweat must be taken into account. It is essential to relate the mineral alterations to the metabolism of nitrogen, or sulfur, or both, and to weight changes. If the alteration in weight represents increase or destruction of muscle tissue, the potassium and phosphorus of the intracellular fluids, which are proportional to the protein, may be calculated. If the weight gain or loss is primarily associated with edema or dehydration, it will be shown in the Na and Cl metabolism and may be calculated as interstitial fluid. If acidosis is present, changes will be found in the mineral cation and ammonium excretion. Calcium and phosphorus changes in the bones cause slight changes in the body weight. The storage of carbohydrate as glycogen may be, for most purposes, neglected. If weight increase exceeds that accounted for by extra- and intracellular fluid increase, the residuum is due primarily to the storage of fat.

### Paths of Excretion

Some insight into the metabolism of the minerals is obtained from a study of the concentrations in the blood serum, for these are related to excretion, as well as to absorption and retention. Thus, if excessively large doses of calcium or phosphorus are fed, it is possible to show that the [Ca] of the blood serum is raised, and it is reasonable to interpret these findings as due to greater absorption. However, such deductions must be made with great caution. The level in the blood does not determine the direction in which the material is flowing. Thus increased [Ca] in the serum may represent calcium flowing from the bones to the urine and feces instead of extra calcium flowing from the intestine toward the bones, and low serum [Ca] may result from rapid deposition in the bones. The mechanisms of the body operate to keep the concentrations of the body fluids nearly constant, and for this reason there

may be large changes in metabolism with small changes in the blood values. Thus on a calcium- or chlorine-free diet the concentration of either in the serum may not be altered from the normal. With increases in the blood constituents, a larger proportion usually find their way into the urine. But high urine values may be found with diminished blood levels.

Compared to the blood, the fluctuations in excretion are very great. There is not a smooth elimination of each element from hour to hour or from day to day, even with constant intake. Therefore not only the concentrations of minerals in the blood and their intakes and retentions must be considered, but also the amounts and proportions of minerals excreted by the various channels.

There has been much confusion in thinking about the fate of ingested minerals. In the older German literature it was customary to consider that the material found in the feces was unabsorbed, and represented that which was not useful to the body. This was subtracted from the total intake, and the amount remaining was said to be absorbed. The retentions were then calculated by subtracting the amounts found in the urine from that which was supposedly absorbed, or utilized, and were stated as percentages of the minerals absorbed. Murlin<sup>70</sup> has discussed the mineral metabolism of infants from this aspect.

Our viewpoint in regard to intestinal physiology is changing, and, although the feces undoubtedly do contain some "residue," or materials which the body is incapable of absorbing, they also contain material which has been excreted or secreted into the gut. The amount of calcium in the feces may be greater than that ingested, hence of necessity excreted or secreted. To differentiate between that secreted and that which was unabsorbed is not at present possible, for minerals may be secreted into small intestine and later absorbed in the large intestine, and *vice versa*. We have taken the position that it is much more simple and conservative to regard material found in the feces as excreted rather than as unabsorbed. On this basis the urine and feces assume equal importance as means of excretion.

Table 40.—Paths of Excretion in the Adult.\*

	Urine		Feces		Apparent
	(% of intake)	(% of excretion)	(% of intake)	(% of excretion)	Retention (% of intake)
Na .....	58	95	3	5	38
K .....	74	79	19	21	8
Ca .....	10	12	73	88	17
Mg .....	35	31	77	69	..
Cl .....	75	98	1	2	22
P .....	47	57	34	43	18
S .....	66	83	14	17	20

\* Calculated from the data in Table 39.

**Distribution of minerals between urine and feces.**—The early studies on nitrogen showed that the amount in the feces was small and fairly fixed, and that the urine content varied primarily with the intake and retentions. This is not true for all the mineral constituents. The paths of excretion are quite different for the different elements in health, and may be altered by various conditions, including disease.

As an example of the normal distribution of minerals between urine and feces, Table 40, calculated from Clark's data on normal men, is given. These percentages are only averages, and represent proportions which are not constant even in normals. The urine contains nearly all the excreted  $\text{Na}^+$  and  $\text{Cl}^-$  three-fourths of the  $\text{K}^+$  and of the sulfur as  $\text{SO}_4^{--}$  and slightly more than one-half of the phosphate. The feces contain five-sixths of the calcium and two-thirds of the magnesium.

Table 41.—Paths of Excretion in the Infant.\*

	Urine		Feces		Apparent Retention (% of intake)
	(% of intake)	(% of excretion)	(% of intake)	(% of excretion)	
	Normal stools <sup>1</sup>				
Fat .....			12		
Protein .....			8		
Total ash .....			40		
Na .....	68	84	12	16	20
K .....	55	78	15	22	30
Ca .....	Trace	..	67	100	33
Mg .....	Trace	..	57	100	43
Cl .....	79	95	4	5	17
P .....	37	50	37	50	26
	Loose stools <sup>2</sup>				
Fat .....			23		
Protein .....			15		
Total ash .....			47		
Na .....	28	43	37	57	35
K .....	38	50	38	50	24
Ca .....	Trace	..	70	100	30
Mg .....	Trace	..	66	100	34
Cl .....	70	24	18	76	12
P .....	36	46	41	54	23
	Very loose stools <sup>3</sup>				
Fat .....			41		
Protein .....			25		
Total ash .....			84		
Na .....	6	5	103	95	-9
K .....	27	24	84	76	-11
Ca .....	Trace	..	79	100	21
Mg .....	Trace	..	100	100	0
Cl .....	17	24	53	76	30
P .....	42	46	48	54	0

\* Calculated from Holt, Courtney and Fales,<sup>45</sup> Table 6.

<sup>1</sup> Seven patients, 11 periods.

<sup>2</sup> Eight patients, 14 periods.

<sup>3</sup> Six patients, 10 periods.

Table 41 shows the paths of excretion of infants under normal and abnormal conditions. It can be seen that the percentage of excretion in the feces of infants is greater than that of adults, for most of the elements. This may be partly accounted for by the greater retentions in infants, for the urine content is more nearly a reflection of intake and retention than the feces. The breast-fed infant produces urine which is nearly free of calcium and phosphate. Hamilton<sup>37</sup> has claimed that, for the premature baby, an irreducible minimum of calcium and phosphorus must be provided for intestinal excretion, which will cause negative balances if sufficient intake is not provided.

**Factors affecting paths of excretion.**—In general, when excess of an element is ingested, the amount excreted leaves mainly by that path which was previously large, so that the proportions are not markedly affected.

Alkalosis causes a shift of calcium and phosphorus from urine to feces. Dehydration and acidosis cause an increase in the excretion of mineral cations and this excess is found almost entirely in the increased amounts in the urine. Diarrhea on the other hand causes increased amounts and a larger proportion of the excretion of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  to occur in the feces, especially in infants, so that negative balances may occur (see Table 41). This does not materially affect the paths of excretion of calcium, magnesium and phosphorus.

**Calcium.**—Aub and his associates<sup>10</sup> have standardized procedures for studying the calcium and phosphorus metabolism with low (0.1 gm., or 5 meq./day), moderate (25 meq.), and high (50 meq.) calcium diets. With the low and moderate intakes the amount excreted in the urine and feces shows considerable variation, but in general about 30-50 per cent of the calcium excreted is in the urine. In these cases the calcium balance is always negative. This variation is shown regardless of whether the phosphorus intake is large or small, or the phosphorus balance positive or negative. With more adequate intakes the urinary calcium decreases to  $\frac{1}{4}$ - $\frac{1}{5}$  of the calcium excretion, and when balances are positive, may be even less.

There is, however, no uniformity. With a low-calcium intake (2-2.5 meq.) Wendt<sup>110</sup> found, in an adult, only 5 per cent of the excreted calcium in the urine. But the total excretion was only 9 meq. Loeb<sup>61</sup> reports 50 per cent in the urine at a level of 23 meq. intake. Both cases had slight negative calcium balances, while the latter was in positive phosphorus balance.

The phosphorus in the urine also tends to be a larger percentage of the total excretion when the calcium in the urine is high than when it is low. Conversely, if the phosphorus intake, and hence the urinary phosphorus, is low, the calcium is also low, causing a rise in the proportion of fecal to urinary calcium.

Most investigators agree that an increased phosphorus intake results in increased calcium excretion. Phosphates excreted in the feces are nearly always accompanied by an increase of calcium. Phosphates in the urine increase the other mineral cations but not calcium. The careful studies of Aub *et al.* have shown that neither acid phosphate nor high-protein diets, plus  $\text{NaHCO}_3$  to offset their acid effect, materially affected the calcium excretion in either urine or feces on low-calcium intakes.<sup>29b, 79</sup> Only when bone salt dissolution is due to an excess of anions other than phosphate ions is extra calcium excreted in the urine.<sup>29a</sup>

The soaps excreted in the gut are one of the factors which affect the calcium excretion. Telfer<sup>100</sup> has found that fatty acid excretion is always accompanied by increased calcium excretion in the feces. This usually means an increase in the total calcium excretion. It is well exemplified in sprue and celiac disease. Herter,<sup>43</sup> and Aub and Farquharson,<sup>5</sup> have shown that large amounts of calcium may be thus lost.

Other factors of importance are the action of vitamin D and internal secretions. Vitamin D causes increased calcium retention in infants with rickets, with a much decreased excretion in feces and a negligible increase in the urine. In normal adults large doses at first decrease the proportion of calcium in the urine, but after a few days this is reversed, and the main effect is to increase materially the proportion of calcium in the urine without altering the total excretion.<sup>11</sup> Parathyroid hormone increases urinary calcium without diminishing that in the feces, and in large doses causes negative balances.<sup>1</sup> Increased thyroid activity causes negative calcium balances and increases both urinary and fecal calcium excretion proportionally (although Aub reported that 56 per cent may appear in the urine).<sup>7</sup> In myxedema on the other hand, calcium excretion is found to be 40 per cent below the average of normals.

*Magnesium.*—The metabolism of magnesium has received less attention than that of any of the other common minerals. It presents many enigmas. Examination of the data show that the amount excreted in the urine and feces is more variable than is the case with other elements. All metabolism studies, either of infants or adults, show a surprising number of negative balances. This is further considered under *Magnesium* (p. 167). Calcium injection causes increased magnesium excretion in the urine, and *vice versa*.

*Phosphorus.*—Clark's data show a slight reponderance of phosphate in the urine over that in the feces, with positive phosphorus balance. In Wendt's experiments two-thirds of the phosphorus excreted was in the urine, with negative balance. Wiley, Wiley and Waller,<sup>113</sup> with a subject in approximate equilibrium, also report about 60 per cent of the excreted phosphorus in the urine.

Loeb *et al.*<sup>61</sup> with moderately low calcium intake, found approximately 75 per cent of the excreted phosphorus in the urine. With low

calcium-high phosphorus intakes, Farquharson, Salter and Aub<sup>29b</sup> found seven-eighths of the phosphorus in the urine. With  $\text{NH}_4\text{Cl}$  in addition, there was only a slight increase in the urinary percentage of phosphorus. Diets high in calcium cause an increase in fecal phosphorus. High calcium-low phosphorus intakes render the urine practically phosphate-free. Phosphorus intakes high enough to cause a rise in the phosphate concentration of the blood serum cause increase of phosphate excretion by the urine. Phosphate given intravenously also is found largely in the urine.<sup>2</sup>

Increased parathyroid activity, although it lowers the blood serum phosphate, causes increase of that in the urine. In normal individuals, when vitamin D causes increased calcium in the urine, phosphate excretion by the same channel is also increased.<sup>11</sup> Recent studies by Albright *et al.*<sup>3</sup> have shown that A.T.10 has less effect on the absorption of calcium than vitamin D, but a greater effect on increasing urinary phosphate excretion (see Chapter 4).

#### PREGNANCY AND LACTATION

##### Physiological Changes

The problems of pregnancy and lactation cover special fields of mineral metabolism and therefore need separate treatment. During the reproductive cycle far-reaching physiological changes take place in the maternal organism.

*Acid-base equilibrium.*—It has been shown that in pregnancy the blood  $[\text{HCO}_3^-]$  is diminished, but this is not to be interpreted as an acidosis. It has been shown, however, that this is accompanied by a decrease in concentration of mineral cations.<sup>56, 57, 58, 74</sup> It has been maintained<sup>72</sup> that there is a true shift in pH toward the alkaline side, but the changes are so small as to be within the doubtful zone, and for practical purposes they may be considered as unimportant. The urine may be alkaline. The serum colloids and lipids show conditions similar to those found in nephrosis.

*Tetany.*—The serum  $[\text{Ca}]$  is known to be low, and tetany may result. Maxwell and Miles<sup>65</sup> found tetany endemic in portions of China, associated with osteomalacia and low-calcium intakes. Maternal tetany is not uncommon in Austria, where other forms are also prevalent, but is rare elsewhere.<sup>54</sup> It occurs in late pregnancy, shortly after delivery, and during lactation. It may be fatal, though often transient or latent. Hartley<sup>40</sup> has stated that although manifest tetany is rare, latent tetany is comparatively common. The symptoms are insomnia, increased irritability of disposition, edema not associated with heart or kidney disease, cramp-like or aching pains in the lower extremities, and a tingling or burning sensation sometimes only in the fingers or toes. Increased

irritability to galvanic currents may also be present. Delayed clotting time of blood and increased friability of the tissues have also been found. All these conditions were greatly improved by large doses of vitamin D or parathyroid hormone, and adequate calcium intake.<sup>76, 89</sup>

The cause of this condition is not clearly understood. Presumably there is no relation between tetany and the alkalosis due to the vomiting of pregnancy, for the vomiting occurs early and the tetany late. Cantarow<sup>17</sup> suggests that parathyroid deficiency may be an important factor. The recent work at Wisconsin<sup>55</sup> shows that pregnant animals after parathyroidectomy require over a thousand-fold more vitamin D to prevent tetany than do non-pregnant animals. Even if tetany is due to an increased need for the hormone, vitamin D therapy is more rational than treatment with parathyroid extract if the maternal store of calcium is to be preserved, for parathyroid acts always by withdrawing calcium from the bones.

Milk fever and grass tetany in cattle are apparently closely related to tetany. Sjollem and Seekles<sup>90</sup> recently found that the [Ca] in the serum was diminished in both, but the [Mg] was increased in the former and decreased in the latter.

*Effect of vitamin D.*—If the maternal store of vitamin D is abundant it is more difficult to produce rickets in the young (of experimental animals). If the mother's intake is deficient in vitamin D, calcium or phosphorus, the young are more susceptible to rickets.<sup>104, 106</sup> Because infantile rickets and tetany are extremely rare before the 3rd-4th month, tetany of infants at birth, whose mothers had tetany during pregnancy, is of extreme interest.<sup>41, 64, 89</sup> Both mothers and infants were cured by vitamin D. Tetany occurring in calves on milk only, is associated with low serum [Mg].

*Teeth.*—Dental defects resulting from pregnancy and lactation have been so prominent as to give rise to the aphorism, "For every child a tooth." It is possible that this type of caries differs from that of children (which has been discussed under *Teeth*, p. 145), because of the relative parathyroid deficiency under this extra burden. Erdheim<sup>28</sup> early showed that parathyroid deficiency was associated with brittle teeth in rats, and in humans it has long been known to be associated with thin and brittle nails, hair, teeth, and also cataracts. The condition of brittle and carious teeth is especially important in pregnancy, and here the results of vitamin D therapy have been especially encouraging. Caries is arrested and the cavities recalcified.<sup>67</sup> Richardson<sup>76</sup> has corroborated these findings. Toverud and Toverud<sup>106</sup> have reported that in dogs the lack of minerals and vitamin D in the diet of the mother resulted in defective teeth in the young. Drain *et al.*<sup>26</sup> found that the progress of dental caries in women could be arrested by supplying a diet which maintained a positive balance of calcium and phosphorus.



*Vomiting of pregnancy* is so general and often serious that further investigation is desirable. The cause in all probability does not lie in the mineral metabolism, but in the endocrine glands, but the condition causes an upset in the mineral and water metabolism. The result may be an acidosis, from ketones or other organic acids, similar to that found in the cyclic vomiting of children, or an alkalosis due to chloride loss. Administration of sugar to overcome the ketosis in the first case and of NaCl to replace lost chloride in the second are desirable, but  $\text{NaHCO}_3$  is contraindicated in both cases.

*Eclampsia*.—This condition is an intensification of the changes found in normal pregnancy. The cause is obscure. The oncotic pressure is definitely lowered; the  $[\text{HCO}_3^-]$  and pH are definitely diminished.<sup>57</sup> The  $[\text{Na}^+]$  in the serum<sup>78</sup> shows the extreme decrease of 14 per cent. The  $[\text{Ca}]$  is usually lowered.<sup>4</sup> The  $[\text{Cl}^-]$ ,  $[\text{HPO}_4^{--}]$ ,  $[\text{H}_2\text{PO}_4^-]$  and  $[\text{SO}_4^{--}]$  in the serum increase.<sup>5</sup> The urine is acid, but the ability of the kidney to form  $\text{NH}_4^+$  is lessened. The blood pressure is increased. Minot and Cutler<sup>69</sup> have suggested a resemblance to liver injury, and therefore sought and found increases in guanidine. In both cases calcium therapy afforded relief. Cantarow<sup>17</sup> has correctly stated that relief by calcium therapy is no indication of the existence of a calcium deficiency or abnormality of calcium metabolism. Richardson<sup>76</sup> obtained marked improvement with vitamin D therapy. Strauss<sup>96</sup> has shown that the degree of hypertension is closely related to water retention.

### Balance Studies and Requirements

*Pregnancy.—Fetus*.—It is obvious that the growing fetus requires its quota of all minerals. Data for fetal composition have been given. The requirement has been summarized as follows.<sup>86</sup> The ash of the fetus contains 1,197 meq. of anionogens (calculating phosphorus as 1.8 eq./mol), and 1,868 meq. of cationogens—an excess of 671 meq. of positive minerals. The mother must supply to the fetus mineral cations as follows: from the 4th to the 5th month, 19 meq.; from the 5th to the 6th month, 100 meq., from the 6th to the 7th month, 374 meq.; and in the last 2 months 1,360 meq. Stated in other terms, for the last 2 months the mother must supply daily 23 meq. of positive minerals and 15 meq. of negative minerals, an excess of 8 meq. of cations per day. These mineral totals equal more than 1 gm. of ash per day, and take no account of the placenta, uterus, or other maternal requirement.

Although the pregnant woman has a special demand for calcium and phosphorus she has no special mechanism for conservation of these minerals. The usual amount of calcium is required for excretion. Bauer, Albright and Aub<sup>10</sup> found that negative calcium balances resulted from low calcium intakes in either early or late pregnancy to approximately the same extent as in normal controls. Therefore the demand for cal-

cium by the growing fetus does not lessen the amount which must be excreted. If the intake is not adequate to provide both for excretion and for the growth of the fetus, the needs of the fetus are fulfilled at the expense of the maternal stores. Sherman has shown that repeated and rapid pregnancies and lactation in rats can lower the calcium content of the maternal body. Virgin rats have a higher, multipara a lower percentage of body calcium than males. Although fetal composition varies but little, recent studies tend to indicate that mothers fed adequate vitamin D and minerals endow their young with a larger mineral supply.<sup>20, 35, 73, 104, 105</sup> Therefore if the pregnant woman is already in a state of mineral depletion, and the intake is inadequate, the fetus will show deficits of calcium and phosphorus.

*Pregnant woman.*—In 1910 Hoffström<sup>44</sup> made a metabolic study of a pregnant woman, for N, Ca, P, Na, K, Cl and S, and this remained the most complete study available until those of Macy and associates.<sup>49, 50, 62</sup> He was able to show from calculations of the composition of the fetal body that the mother retained enough minerals to satisfy the needs of the growing fetus and to store additional material. Macy *et al.* have shown, in agreement with Hoffström, that the pregnant woman retains a great deal more of each of the minerals than is required for the fetus. Their study constituted continuous balance measurements over the last 140 days of pregnancy, and also extended into the periods of lactation and post-lactation. Their subject, whose diet during pregnancy contained 122 meq. of excess cationogens per day, retained 34 meq. of excess mineral cations per day. This is much greater than the daily cationogen-excess balance of the fetus. These data are in agreement with those of Coons *et al.*<sup>22</sup> who showed in 20 balance experiments an average retention of 43.6 meq.  $\pm$  2.8 of cationogen-excess per day. Macy calculated that the following percentages of the mother's retentions were supplied to the fetus:

Na 6.8	Cl 5.8
K 2.3	P 40.3
Ca 46.0	S 1.6
Mg 5.2	

The retentions of sodium and chlorine and also potassium as measured by this method indicate that in the last half of pregnancy the woman stored amounts of these elements more than equal to the whole body content. Inasmuch as sweat was not taken into account (see also p. 334) these values cannot be interpreted as stores added to the maternal body, and await further interpretation. The fate of the large magnesium retentions which were uniformly found on an intake of 0.6 gm. per day has not received adequate critical analysis. Magnesium intake of over 0.4 mg. per day is necessary to prevent negative balances.

The total accumulated calcium storage of 50 gm. and phosphorus of 37 gm. provide large excess over the requirement of the fetus. The excess calcium is stored in the bones with part of the phosphorus. The rest of the phosphorus, and the nitrogen, sulfur and some potassium allow extra protein formation. These reserves may be drawn upon during the lactation period.

The calcium intakes were 1.5-3 gm. and the phosphorus 2.0-2.7 gm. per day. Adequate vitamin D was given. There was no increase in the intake as the end of pregnancy approached. It is felt that these amounts are liberal but not excessive. Coons<sup>21</sup> found both positive and negative balances on intakes of 1.4 gm. of calcium and 1.6 gm. of phosphorus. Toverud<sup>104</sup> found negative balances common in late pregnancy and positive balances only with calcium intakes of 1.6-1.8 gm. To obtain satisfactory balances vitamin D is required. Four hundred units of vitamin D and 1 qt. of milk daily should be included in the diet of pregnant women. Sandiford *et al.*<sup>80</sup> found that average intakes of calcium, phosphorus and magnesium were smaller than those given above. In pregnancy as in other conditions it has been shown that on a given intake the retentions will be much greater if there has been a previous stringency in the diet.<sup>49</sup> The acid- or alkaline-ash value of the diet was shown by Goss and Schmidt<sup>35</sup> to be a minor factor. The diet has an alkaline-ash value when milk is a liberal component.

**Lactation.—Women.**—Although positive calcium balances are the rule in pregnancy, such is not the case in lactation.<sup>16, 63</sup> Macy and associates measured for the first time the cationogen-excess balances during lactation and showed that with intake of 135 meq. excess of positive minerals per day there was a mean retention of 47 meq. = 42. Sodium, chlorine and potassium balances which were positive, as in pregnancy, are subject to the same difficulty of interpretation and account in large part for the positive cationogen-excess balance. In spite of an intake of 3 gm. of calcium, negative calcium balances occurred and the urine became practically calcium-free.<sup>50</sup> Magnesium balances were negative with an intake of 0.67 gm., as were phosphorus balances on 2.8 gm., and sulfur on 1.4 gm.

It seems therefore that losses during lactation occur in spite of very liberal intakes. For as long as a year after cessation of lactation in women negative calcium (but not phosphorus) balances continued.<sup>25</sup> The difficulty has been attributed to the inability of the intestine to absorb enough calcium even on high intakes, because calcium is lost in the feces. This might be due equally to re-excretion. No one, so far as the author is aware, has shown that the negative balance is due to a limited ability to absorb minerals. Macy *et al.*<sup>63</sup> showed that a daily supplement of 15 gm. of cod-liver oil plus 10 gm. of yeast exerted a favorable influence on calcium and phosphorus retentions when given

to lactating women. The calcium balance became positive in one case, and the negative balance diminished in two others. Phosphorus balances became positive. As comparable experiments have not appeared where the vitamins were given separately or in larger amount, or during pregnancy, these investigations as far as humans are concerned must be considered as important pilot experiments.

Many have advocated feeding calcium during pregnancy and lactation, usually in the form of calcium lactate or carbonate. Such additions in Forbes' experiments resulted in a ratio of Ca/P of about 3/1 in the diet. We know from experience with experimental rickets that such a disproportion, in the absence of vitamin D, causes lower retentions of both calcium and phosphorus. Therefore it seems reasonable that the Ca/P of the feeding should approximate that of milk, about 1.3/1. The effect of such ratios should be tried experimentally at various levels or concentrations.

A generous diet, fortified with large quantities of vitamin D, and liberal additions of B<sub>1</sub>, B<sub>2</sub>, and C, might sufficiently increase the storage during pregnancy so that lactation would not cause depletion beyond normal, or might maintain the calcium reserves throughout. Garry and Stiven<sup>33</sup> suggest that the normal requirement should be supplemented by additions of at least four times the calcium secreted in the milk and sufficient vitamin D to prevent losses during lactation. The calcium intake, preferably as milk, should amount to approximately 2 gm. of calcium per day or more.

*Dairy animals.*—The problem of lactation is most intensified in the dairy industry, where physiological success and financial success are parallel, and the statement of Theiler and Green<sup>101</sup> that the cow "draws upon its convenient skeletal bank however high its dietary income may be," is extremely apt. Milking-cows showed considerable negative balances until late pregnancy when the milk production was small. This condition could not be reversed by the addition of any mineral supplement.<sup>27, 31</sup> The effects of ultraviolet light and vitamin D on mineral balances of lactating goats and cows have been carefully studied by the Wisconsin group. More favorable results were obtained with goats than with cows.<sup>39</sup>

In the light of these findings the question arises as to whether negative mineral and especially calcium balances are inevitable or deleterious. Studies on cows have shown that as much as 20 per cent of the body calcium may be lost over a period of years without injury. When the loss is greater than this, fertility and milk supply are diminished. Forbes states that the only way out is to allow longer resting periods and more liberal feedings between pregnancies, or the animals are stunted in growth and less successful in rearing calves and in production of milk.

Goss (cited by<sup>82</sup>) found that, with repeated pregnancies and low calcium intakes, rats could lose 60-70 per cent of their body calcium.

### MINERAL REQUIREMENTS

The adequacy of the food intake can be considered only in relation to the individual consuming it. Therefore any alteration in his metabolism due to disease must be taken into account. However, the greatest single factor that has caused difficulty in evaluating intakes is, in our judgment, the previous condition of the individual, as mentioned on page 323. It is only through such long-time studies as those of Clark and of Macy that one can appreciate over what long periods deficiencies or excesses may show their effects. This explains in large measure the great variability in the data of various investigators, as to the adequacy of given intakes.

The mineral intake may be described in terms of calories, grams, or specific foods, but the requirement for intake must be defined in terms of the body. Energy metabolism is proportional to activity, but mineral metabolism is not, and therefore mineral requirement cannot be based upon caloric requirement. Standards may be derived from height measurements, but such values are related with difficulty to the composition of the body and organs. Within different age groups, amounts may be mentioned in terms of "per person per day," and for the adult an average weight of 70 kg. is assumed. Requirements are much more accurately stated for infants and children as amounts per unit of body weight. This gives a more rational standard, because the body and its parts all have a definite content of minerals. Further, because growth is coördinated, the fat-free organs and systems are in approximately fixed proportion to the total body weight. For practical purposes we have used the gross weight, and expressed requirements as meq. or gm. per kg. per day.

There are two aspects to the problem of mineral requirements: first, the requirement for maintenance of equilibrium in the body, and secondly, the requirement for optimal growth. The three principal methods for determining these requirements are:

1. The accumulation of statistical evidence as to the average intakes of large groups. This gives information only about the order of magnitude of intakes necessary for maintenance.
2. Balance studies on individuals with different intakes and under different conditions. This method gives valuable information concerning the amounts of minerals required for maintenance, and the factors which affect requirement. Single studies give little insight into the requirements for growth over the whole period of infancy and childhood, but they do supply data as to the proportion of ingested minerals

which can be retained, as well as establish standards below which no retentions are possible. Statistical treatment of large numbers of such studies on normally growing children offer the best means available at present for the determination of requirements for growth.

3. The calculation of accretions necessary to permit the normal composition of the body at different ages. This can be applied to the study of requirements for growth. See Chapter 2, page 48.

From the previous discussion of the intakes of groups in this and other chapters it is obvious that, as a rule, few if any problems arise from inadequacy in the diet of Na, K, Cl, S or Mg. For growth there is need for so little sodium and chlorine that there is never any stringency of these elements in the diet, although liberal intakes are desirable. Only 1.0 meq. (or 23.0 mg.) of sodium and 0.7 meq. (or 24.5 mg.) of chlorine are necessary to supply ions for the interstitial fluid in the one ounce of substance which a baby adds to its weight each day. The potassium content of natural foods seems adequate at all times. If the protein intake is adequate the sulfur requirement is met. Studies of magnesium balance at all ages show great variability (see *Magnesium*, p. 167). Iron, iodine and traces present special problems and have been discussed in their respective chapters, so will not be considered here.

Calcium and phosphorus are important not only in relation to bone growth and development, but to the growth and well-being of the whole body. Considerable amounts are necessary to prevent depletion. This demonstrates the wisdom of the emphasis which Bunge, Sherman and many others have placed on the adequate consumption of these minerals. Therefore the main problem in mineral requirement centers about calcium and phosphorus and iron, and it is quite proper that more work has been devoted to them than to all the others combined.

### **Food Factors Affecting Requirement**

We have to remember that we eat natural foods containing both known and unknown substances, or as Sir F. G. Hopkins succinctly stated: "We thought we were feeding our animals proteins, fats and carbohydrates, but what we were really giving them was carrots and oats." Before defining the requirements for mineral intake in terms of grams of calcium and phosphorus, it is necessary to note some of the food factors which affect the utilization of the minerals. These fall into two classes: the relation of other food constituents to the minerals, and the relation of the minerals to one another.

The concentrations of the minerals ingested and the time relations with regard to food ingestion, apart from the total amounts of minerals, may markedly alter their utilization. It has been shown, for example, that calcium salts administered in the post-absorptive state cause a

marked and prolonged increase in the blood calcium, which is absent when the same dosage is given with food.<sup>77</sup>

Considerable evidence has accumulated that calcium from different sources is not equally well utilized, and that that of milk is retained better than that of vegetables. Recent experiments have shown that the high oxalate content of spinach renders it practically useless as a source of calcium (and perhaps of iron).<sup>30</sup> The better utilization of calcium when fruits are liberal may be due to their vitamin content, or their bulk, or to their citric acid or other organic acid content.<sup>18</sup> When crude fiber is too great, decreased utilization of calcium is demonstrable.

The dietaries of Asiatics contain little or no milk, and it might be thought that for this reason they would be deficient in calcium and phosphorus. However, they utilize many bones which we reject. Rubner has stated that the diets of the Japanese are adequate although low in calcium because they are also low in fat. High-fat diets cause an increase in the calcium excreted in the feces, and hence result in lower retentions. If fat is sufficiently high in relation to carbohydrate, it causes the production of ketone bodies and acidosis.

Carbohydrate also bears relationship to the utilization of calcium. In parathyroidectomized animals on a diet so low in calcium that tetany ensues lactose in liberal amounts will prevent tetany, whereas other carbohydrates will not. Further, with high-lactose diets calcium additions are toxic.\* Mellanby has claimed that phosphorus is not so well utilized in the presence of cereals. (See p. 187.)

If the total caloric intake is insufficient the body must burn its own stores of fat and protein. So too, a diet insufficient in protein causes destruction of body protein. Thus minerals are freed, and acid end products are produced. When a sufficient excess of anions is present, from whatever source, calcium is withdrawn from the bones and excreted unless supplied in extra amounts.

**Calcium-to-phosphorus ratio.**—In recent years, largely as a result of studies in experimental rickets, we have learned that the interrelation of minerals may be more important than the actual amount of any single element. The ratio of Ca/P in the diet has been shown to be of considerable importance. A diet which permits normal deposition of minerals in bone may be transformed to a rickets-producing diet by increasing the calcium while maintaining the phosphorus constant. A normal diet may be altered to one that causes rickets by diminishing the phosphorus content. Under these conditions the retentions of both calcium and phosphorus are impaired, for the materials are best retained in approximately the same proportions as those of the food, but will not be so retained unless the ratio is that required for bone formation.

\* Dr. H. S. Mitchell, personal communication.

Thus, it is possible to increase not only the phosphorus but also the calcium retentions by additions of phosphate to a diet low in phosphorus. Such experiments demonstrate the futility and even injury that result from attempting to increase the calcium balances by increasing the calcium intake alone. The same effects are demonstrable not only when the Ca/P ratio is high, but when any of the heavy metals prevent the utilization of phosphorus by the formation of insoluble phosphates. Similarly, when the calcium is low and the phosphorus is high, the utilization of both is impaired. These experiments have been made largely upon experimental animals because infants are given milk: that is, the Ca/P is a constant ratio. The excellent experiments of Orr, Holt, Wilkins and Boone,<sup>75</sup> however, show that the same underlying principles apply equally to children.

Animal experimentation has shown that, regardless of the ratio, diets become more or less ricketogenic as the absolute amounts of calcium and phosphorus are lowered or raised. The important thing for human nutrition is, therefore, that the intake of each should be adequate. If this is done neither will be in sufficient preponderance so that ratios need be seriously considered. The decreasing ratio of Ca/P intake from infancy to adult life can be computed from the data in Tables 37 and 38. For infants the intake requirement may be stated in amounts of milk, for this is the preponderant foodstuff. For children the requirements for calcium and phosphorus are most easily met by including a liberal allowance of milk, of which the content of both calcium and phosphorus is not only high but also in good proportion and readily available. The standards for calcium and phosphorus intake are given on the assumption that adequate vitamin D is also given, but if the mineral intake is insufficient the retentions will not be adequate, vitamin D or no vitamin D.<sup>95</sup> (See further under *Rickets*.)

### Calcium and Phosphorus Requirements for Maintenance

At all ages from the premature infant to the adult, considerable intakes of calcium and phosphorus are required to maintain the constant flux to and from the bones, and to provide for the necessary minimal excretion.

Extrapolation of the data of Jeans, Stearns *et al.*<sup>52</sup> shows that normal artificially fed babies require intakes of 50-60 mg. of Ca/kg./day to maintain equilibrium. Wang *et al.*<sup>109</sup> found that children 8-11 years of age, without exception, had positive balances with intakes of 23 mg. of Ca/kg./day, and negative balances with intakes below this value. From analysis of 97 experiments on adults Sherman<sup>83b</sup> found that equilibrium could be obtained on approximately 0.45 gm. of Ca/man/day (22.5 meq.) or about 7 mg./kg. This value represents the lowest level at which balance can be approximated in a normal man. In fact, Loeb and asso-



ciates<sup>61</sup> found that with this intake their normal subject was in slight negative balance without exception for 40 consecutive days. Leitch<sup>59</sup> analyzed 400 balance experiments on women and found that about an equal number of positive and negative balances were obtained with 0.55 gm. of Ca/day, or 9-10 mg./kg.

Calculations for phosphorus from the data of Jeans *et al.* on infants show that 45-50 mg. of phosphorus per kg. per day are necessary for maintenance of equilibrium. The children studied by Wang *et al.* had positive phosphorus balances with intakes of 35 mg./kg./day and negative balances with lower intakes. From an analysis of 95 phosphorus balance experiments Sherman has given a minimum requirement of 0.88 gm. of phosphorus per man per day (28 mM), or 12-13 mg./kg. This is in close agreement with the value of 0.91 gm. which Wendt<sup>111</sup> gives as his standard, based upon the relation to protein requirement.

It is evident that the maintenance requirement for both calcium and phosphorus, per kg. of body weight, diminishes with increasing age.

As a result of inadequate intake, negative balances of calcium and phosphorus may continue for very long periods of time in the adult. It is only after a period of 5 or 10 years or longer that such shortages manifest themselves in gross pathological changes. On the other hand, increased intakes may enable deficiencies to be made up slowly over a long period of time, as is shown by Clark's subjects, who showed positive balances for seven months.

Sherman allows an excess of 50 per cent over his experimental minimum for a standard of intake of calcium and phosphorus for adults. This sets the calcium requirement at 0.70 gm., or 35 meq./man/day, and the phosphorus requirement at 1.32 gm., or 42 mM.

### Calcium and Phosphorus Requirements for Growth

Retentions can take place only when excesses over requirements for maintenance are supplied. The calcium and phosphorus requirements of infants and children therefore include allowance not only for maintenance but also for growth. Practically, intakes so low as not to allow for retention are rare in infancy (except for the premature infant), but become increasingly common during childhood, in spite of the fact that the quota for growth is maximal in infancy and declines throughout childhood to zero as adulthood is approached.

If we assume that breast milk is ideally adapted to the needs of the infant for growth, then there is no problem of mineral requirement. But the developments of nutrition during the last quarter-century have shown this mineral intake to be sub-optimal. The data on calcium and phosphorus retentions of infants and children can now be considered with the purpose of determining standards for growth.

Analysis of the data given earlier in the chapter shows that the

absolute amounts of calcium and phosphorus retentions are roughly in proportion to the intakes, but that the percentage of intake retained decreases with age. Whatever the requirement for maintenance, the total intake, and not just that in excess of maintenance requirement should be used as a basis for calculating retentions, for only when the intake is extremely low or extremely high is there any alteration of the percentage of the intake retained, at a given age.

The data show that the breast-fed infant is a law unto himself. With a calcium intake of only 45 mg./kg. he retains about 40-60 per cent, or 20 mg./kg./day. The phosphorus intake is 25 mg./kg., with average retention of 55 per cent, or 14 mg./kg. These infants, in spite of low intakes and retentions, rarely show rickets.

The artificially fed infant on the other hand, retains only about 33 per cent of the calcium ingested (see Table 41), but his intakes are high, and therefore with a calcium intake of 120-180 mg./kg./day, his retention may be two or three times that of the breast-fed infant. It is judged that 120 mg./kg. constitutes an adequate intake. Infants retain about 25 per cent of phosphorus consumed, and an intake of 95 mg./kg./day is considered adequate. It is assumed that adequate vitamin D is given with these intakes. The higher retentions obtained by the Iowa investigators<sup>51</sup> are due both to liberal vitamin and to diets with larger than usual mineral content. It should be pointed out that their babies exceed the average values for increase in both height and weight.

It is more difficult to determine requirement for children than for infants, for the data are less numerous and more variable. It seems best to base the standard for requirement on intakes which have been shown to result in satisfactory retentions. Both highest and lowest values should be avoided. The average represents the judgment of the past generation as to the proper intakes. The data in Tables 37 and 38 have been culled from the literature by Macy, and may be summarized as follows. The intakes of calcium per kg. decrease with age from 70 mg./kg. at 3 years to 12 mg. at 16 years, which may be compared to the adult maintenance value of 10 mg./kg. (See also <sup>23a</sup>). Corresponding amounts of phosphorus are 79 mg./kg. at 3 years, and 35 mg. at 16 years, compared to the adult value of 19 mg./kg. When these are computed to total intakes they give values of about 1 gm. of calcium and 1.3 gm. of phosphorus per child per day throughout the whole period. Therefore children have a larger total requirement than adults, for their size increases faster than their per kg. requirement decreases, and although at puberty they are of large size, they still need minerals for storage as well as maintenance.

Differing from infants, and similar to adults, the intake of phosphorus is in excess of calcium throughout childhood. However, inasmuch as children from 8-12 years old show retentions of calcium of more than

25 per cent of the intake, and of phosphorus, 15 per cent, the ratio of retention averages  $\text{Ca/P} = 1.5/1$ , or less. The older group of children show a reverse in this ratio, for they retain only 15 per cent of calcium intake, and 20 per cent of phosphorus. This represents the older child's proportionately smaller need for building of bone and greater gain in muscle.

**Comparison of standards.**—The calcium retentions, calculated from the composition of the body at different ages, given in Table 8 (p. 50) are lower throughout than the values shown in Macy's data, Table 37, except as puberty approaches. This indicates either that the diets selected for children on metabolic studies were better than average, or that these children were below average before the experiments, or that the standard given in Chapter 2 is too low. However, the difference between the two does not constitute a great discrepancy. By allowing for a more rapid rate of growth in the early years and less in the later, in the calculated data, the two could be brought into close approximation, and would represent as close an agreement as could be expected from two such divergent methods of approach. At the present state of our knowledge the standards given by any method offer only rough approximations.

There now arises the question: Is the calculated standard sufficient, or do the experimental data offer a better standard, and if so is this optimal? There are two opposing viewpoints. The first is that we should strive to obtain higher retentions, and that maximal retentions are optimal. This has been the thesis of Sherman,<sup>84</sup> who claims that larger calcium retentions lead to increased rate of growth and an extension of the period of mature life. The second is that well-being is promoted by slower growth. McCay<sup>66</sup> states that stringency in early life leads to prolongation of life; his animals were still vigorous at 1000 days of age, when Sherman's high calcium-fed rats had died. This is a question which cannot be determined *a priori*.

Low calorie intake with adequate minerals results in a tall but lean individual. Extra calories give fat but no added height. Slightly low calcium and phosphorus intake may not prevent growth, but during such growth the bones contain less calcium, and the chemical maturity of the skeleton is delayed. Extreme inadequacy of calcium and phosphorus result in a stunted individual.

No one so far as the author is aware has demonstrated harmful effects from high retentions in children fed natural foodstuffs. Pathological calcification does not take place. It is not known whether long-continued high intakes result in stone formation or degenerative diseases such as arteriosclerosis.

The author feels that the middle ground is the safest. The standards given in Chapter 2 probably represent sub-optimal values. The analysis of the skeleton on which the calculations are based comes from an era

when rickets and anemia were physiological. It has been our experience over the last two or three generations that the performances of growth in height and weight of each generation have exceeded those of the previous one. It is the unavoidable belief that this increase represents an increase in well-being, and is a result of our better understanding and practice of the fundamentals of nutrition.

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Various salts act differently. The anions follow the Hofmeister series as diuretics. Sodium is associated with water retention. Calcium and potassium are diuretics. Acids remove water from the body and alkalosis is associated with edema. Alkaline salts in acid.